

nature

Volume 247

February 15, 1974

Is it fair to force your baby to smoke cigarettes?



This is what happens if you smoke when you're pregnant.

Every time you inhale you fill your lungs with nicotine and carbon monoxide.

Your blood carries these impurities through the umbilical cord into your baby's bloodstream.

Smoking can restrict your baby's normal growth inside the womb.

It can make him underdeveloped and underweight at birth.

Which, in turn, can make him vulnerable to illness in the first delicate weeks of his life.

It can even kill him.

Last year, in Britain alone, over 1,500 babies might not have died if their mothers had given up smoking when they were pregnant.

If you give up smoking when you're pregnant your baby will be as healthy as if you'd never smoked.


The Health Education Council

...or force your wife to feel guilty?

WITH the recent publication by the Health Education Council of the above poster, we return with some reluctance to the smoking and pregnancy issue, subject of the leader of September 14, 1973.

The results of a large study showed that non-smokers have, on average, heavier babies than do smokers, and, probably as a consequence, the pregnant mother who smokes has a 30% greater chance of losing her child than does the non-smoker. Furthermore those who voluntarily gave up smoking during pregnancy had 'non-smoker' babies—heavier and more likely to survive.

The question we raised then and we raise again now is that of the advice that scientists should give the public. It is a central question, and one which crops up in many guises whenever scientific work impinges on public policy making. The obvious first reaction is 'if it abates, do it immediately: discuss cause at leisure'. Dr Renwick has recently argued eloquently for greater emphasis on abatement in medical science (246, 114; 1973). There is, of course, still some residual scientific

disagreement (as our correspondence showed) about whether it is the smoker or the smoking that leads to lighter babies, but surely with 1500 lives a year at stake the Health Education Council is justified in spending £120,000 abatively on a frontal (well, lateral) assault on the smoking mother-to-be?

Clearly, stopping smoking during pregnancy is a most desirable thing. If the campaign had been simply to encourage those for whom smoking is an optional activity to opt appropriately, one could have few quarrels with it. But the approach is more than suggestive. Words like 'force' and 'kill' are intended to shock and the target is as much the hardened as the casual smoker. The back of the leaflet consists of a guide to stopping smoking that could have been culled from an army manual—from "there's no painless way to stop smoking, no wonder cure" through to "having made the decision to stop, you must stick firmly to that decision". Poor pregnant mother. As if she doesn't have enough kindly advice and isn't already under enough pressure without being instructed to give up what may have been a lifelong habit. If she succeeds, it won't (as they say) be painless. If she fails, the campaign will at least have instilled guilt, and if she fails and then loses her child, who is to explain to her that only one in four of such losses is attributable to smoking?

Half a million leaflets have been distributed to await the pregnant mother. This well-intentioned campaign would have been much more effective and humane if the money had been used on half a million somewhat less scaring leaflets for schoolgirls informing them that starting smoking now could lead to problems in pregnancy. The way to advise the pregnant mother is not through a stark leaflet but through a well-informed doctor or nurse who can assess what sort of approach is suitable for each individual—and possibly avoid words like 'kill'.

100 years ago



WE have received some interesting notes of the work done by the eminent Russian explorer, Dr. von Mielucho-Maclay, which we hope to publish next week. Contrary to the advice of everyone, this intrepid traveller and true devotee of Science is determined upon again visiting the east coast of Papua. When his researches here are complete he intends to visit some of the islands of Polynesia and certain parts of the coast of Australia. This, he calculates, will take up five or six years. The Governor of the Dutch East Indies, like a true man of Science, had given Dr. Maclay, for the last six months, roomy and comfortable quarters in his palace at Buitonrov. It would be well if all in high position would imitate this kind of "patronage."

From *Nature*, 9, 311, February 19, 1874.



international news

More planning needed for North Sea oil

Eleanor Lawrence

ALTHOUGH North Sea oil is indisputably the best thing that has happened to Scotland for a long time, the rush to secure sites on which to build drilling platforms has highlighted just how woefully inadequate are present planning procedures.

Only the most extreme preservationists would deny the importance of getting the oil ashore as quickly and as efficiently as possible. As quickly as possible may, however, have quite different meanings to a government facing a worsening economic and political situation and to the concerned individuals and local associations who want full and detailed inquiries to preserve the essential quality of life and environment.

Present planning procedures are ripe for reform say some Scottish conservationists, pointing at the present chaos on the west coast. Dr W. R. Bourne of the University of Aberdeen says that present procedures are wasteful and inefficient and that the only winners at the moment are the lawyers who seem to be *de rigueur* at every inquiry. Most of the sites on the west coast are extremely isolated and far from expert help, so each case must be fought separately and at great expense by the local community.

The present system of separate public inquiries is throwing an increasing burden on the slender resources of local people and local conservation societies.

According to Dr Bourne, money that is urgently needed for positive conservation work, to buy land and develop nature reserves and to clear up the effects of pollution, is being diverted in a steady stream into the pockets of the lawyers. To the development companies the long months of uncertainty, waiting for a decision, can also cost money and sometimes results in lost orders.

Last week Mr Gordon Campbell, Secretary of State for Scotland, presented proposals for a Bill to be previewed to Parliament in the near future. This would enable the government to speed North Sea oil ashore by compulsory purchase of the necessary land, which would then be leased to the development companies. These unprecedented measures seem to have been precipitated both by the energy crisis and by the impasse that has been reached at the

Loch Carron site on the west coast of Scotland, near the Kyle of Lochalsh. The government promises that it would use its powers only in limited cases, mainly to speed up the building of the deep water concrete drilling platforms which are apparently essential to the oil effort.

These platforms have to be built in deep sheltered water with a nearby shore site. Such sites are rare in Britain but there are several around the north-west coast of Scotland, notably Loch Carron, Loch Broom and Dunnet Bay in Caithness. The Secretary of State for Scotland said that the Bill would permit an early start on work at Loch Carron, probably this summer instead of next year as had been expected if permission had been granted after the inquiry.

An added dimension to the battle at Loch Carron is that the site is owned by the National Trust for Scotland, held 'inalienably' for the people of Scotland. A special Bill in Parliament would



○, Sites under consideration for development; ●, sites where development is already well advanced.

in any case have been needed to allow development on such a site and the government's proposals will give them blanket powers over other such sites in any part of Britain.

The government promises that it will take basic responsibility for the site and Mr Campbell promised that there would be provision in the Bill for eventual restitution and restoration of the site. But, as Dr Bourne points out, this becomes a mockery if the government will not allow sufficient time for detailed reports of the present conditions, both social and environmental, to be prepared. At Loch Carron, which will probably be the first victim of the new

legislation, no satisfactory reports of the natural history have been prepared says Dr Bourne. Very little is known about the area as yet but reports coming in at the end of last year suggest that the Kyle of Lochalsh is probably one of the main feeding grounds of the eider duck, a characteristic sight in Scottish waters. Flocks about 2,000 strong have been reported and if building goes ahead the eider duck will certainly be dispossessed.

Professor C. H. Gimingham, professor of botany at the University of Aberdeen and secretary of the University's Environmental Liaison Group fears that, despite government promises, the new proposals will not allow proper time for objection and will only reveal the complete lack of any overall planning for oil development in Scotland.

Under the present procedures, once a development company has found a site, it seeks planning permission in principle from the local County Council. The applications are then considered by the council, which should allow time for objection. If overwhelming objections are received the decision is deferred and a public inquiry set up at which the objectors put their case. Public inquiries can drag on for many months, as in the case of Loch Carron and eventually the Secretary of State for Scotland has to step in.

One serious drawback of the public inquiry system as it stands, says Professor Gimingham, is that only one case can be dealt with at a time. They are, therefore, completely unsuited to the present situation on the west coast which urgently needs an overall planning policy. An instance of the sad situation which obtains at the moment is the case of Dunnet Bay in Caithness. Dunnet Bay is designated as an area of outstanding natural beauty. Last year the American Chicago Bridge group applied for permission to build deep water oil drilling platforms there. Local residents and the Natural Conservancy fought the proposals but lost to the development company's appeals to the national interest and the need for urgency. A few weeks after permission had been granted the company announced that it was pulling out of Dunnet Bay to a site in Ireland, where 'geophysical conditions' are more favourable, and planning permission is expected to be granted in a matter of weeks. This has made people not unnaturally wary of further appeals to the national interest

by both developers and government. The permission to develop remains, of course, and may be taken up at any time.

Professor Gimingham considers that the involvement of National Trust land at Loch Carron is especially worrying and could set a dangerous precedent. In his opinion, a proper planning policy for the west coast should have been able to avoid this.

The Nature Conservancy is also investigating whether the National Nature Reserves which it owns or administers will be covered by the new proposals. The lack of an overall planning strategy is also stressed by Mr R. Goodier of the Scottish Nature Conservancy. Local planning departments have not been expanded to meet the unprecedented demands now placed on them. The present crisis is coming at just the wrong time, he said, as Scotland is entering its reorganisation of local government. A massive injection of new planning talent is needed, he said, but how this is to be achieved without by-passing the essentially democratic procedures at local level is a problem.

The Nature Conservancy opposed the plans to develop at Dunnet Bay and now will probably have to fight its case again when the next development is proposed. Despite the expense and time involved the Nature Conservancy obviously wishes to fight such proposals in the interest of Scottish conservation generally. A proper plan for Scotland would make its task and that of the many other organisations much easier.

Science journals in a prices jungle

John Hall

THE rising prices of scientific journals, combined with the proliferation into ever narrower specialities, is currently a matter of concern to university librarians and all who find themselves faced with subscription rates which bear little relation to the rates they budgeted for when they first subscribed.

The question of cost-effectiveness is an almost impossible one to answer for scientific journals. Not only do journals have very varied reputations and quality standards but the cost of a journal is often an elusive quantity. The advertised prices for a set of journals in 1973 and 1974 are shown in the table. Some journals sell at a single price, others have privileged rates for individuals and yet others published in the United States (not included in the table) have page charges that authors are expected, but not required, to pay. These latter are clearly good value in Europe because Europeans traditionally do not have page charge budgets and thus get a subsidised journal.

The 1973 price per page of learned journals (excluding those such as *Nature* which carry advertising and thus can subsidise their editorial pages with their advertising revenue) is roughly £14 per thousand pages. But when a reduced subscription is available for individuals or society members the price for libraries can be doubled. So, presumably, it is the libraries who subsidise the bargains with which individuals can line their offices.

The question of price rises is not a simple one, as subscribers are asked to pay in advance for an item the quality and quantity of which is not guaranteed to last the year. Thus a modest or zero price rise can reflect a publishing decision to produce less or take less care over it. On the other hand an abstracting journal, riding on the back of a still expanding literature, may have no option but to charge more.

A record breaking increase of 261% in the price of *International Pharmaceutical Abstracts* (published last year by the American Society for Hospital Pharmacists at £16.60 and this year at £58.20) was one of a series of price rises which have prompted the Association of University Teachers (AUT) to launch an inquiry into the costing of science publishing. The AUT has taken a batch of suspected offenders to independent publishers, who are to be asked for realistic estimates of production costs. The union's general secretary Mr Laurie Sapper suspects that certain publishers may be making excessive profits (not a specially untrusting notion, since author's copy for these journals comes as free as God's air) and if the independent costing exercise bears out the suspicion he plans to confront the publishers with the evidence of their greed. Which is all very well, except that the publishers might be expected to know already the extent of their profits and to have reconciled their consciences to an appropriate figure. Unfortunately there is no British Standard specifying the demarcation between excessive and reasonable profit.

There is, however, no denying that the profits from scientific publishing generally look pretty healthy, with some falling into the positively exuberant bracket. Alec Anderson, librarian of Heriot-Watt University, Edinburgh, surveyed price increases of scientific books and journals in his library and found that during the autumn term periodicals cost on average 23.5% more than in the previous year. Apart from the famous increase decreed by *International Pharmaceutical Abstracts*, he quoted the United States *Current Contents: Life Sciences* with an increase of 90% (£48.30 to £86.95); the American Chemical Society's *Chemical Abstracts* (£850 to £1,110); and the *Transactions of The American Association of Electrical and Electronic Engineers* (£252 to £321). Lesson: learned societies may be no more averse to a fast buck than is the commercial operator.

The Library Association has published a price index for periodicals (100 in 1970) which now stands at 147.6; it warns that the index could reach 185 within a year. And with books and periodicals rising in price at something like one and a half times the rate of university costs generally, one or two consumers have balked at the increases. *The Times Higher Education Supplement* had Salford University Librarian Charles Bubb talking about large libraries being vulnerable to milking by publishers and calling for an inquiry into the increased diversion of taxpayers' money to this area of spending. In the same issue the librarian of the University of Birmingham Kenneth Humphries identified the problem of the increased influx of European publications caused by the floating of the pound. Most of these imports were a third more or even double their price three years ago.

A more specific attack on the quality of the products which flood the market was mounted in a letter from an international group of chemists, sent to *Nature* and other journals in the field. British professors Jack Linnett (University

TABLE 1 Comparison of journal prices
(in pounds sterling; concessionary prices in brackets)

Journal	Number of pages	Price	
		1973	1974
<i>Deep Sea Research</i> (Pergamon)	1150	34 (6)	34 (6)
<i>Geophysical Journal</i> (Royal Astronomical Society)	2000	50 (8)	90 (8)
<i>Journal of Fluid Mechanics</i> (Cambridge Univ. Press)	5000	57.50	57.50
<i>Journal of Molecular Biology</i> (Academic Press)	7000	99	141.30
<i>Journal of Physics</i> (Institute of Physics)	2300	39 (10)	42 (10)
<i>Philosophical Magazine</i> (Taylor and Francis)	2900	43	47

of Cambridge) and G. Wilkinson (Imperial College, London) are among eleven academics who point out that the rash of new chemistry journals appearing subsist on high subscription rates rather than publication charges from the authors. They believe that some of the new journals, with a vested interest in building up volume to maintain themselves, have refereeing standards which are lax, to say the least (a certain journal in the field of inorganic and nuclear chemistry has "minimal refereeing standards", and contains "stuff which ought never to see the light of day", says Professor Wilkinson).

The chemists see over-specialisation encouraging the proliferation of journals and being in turn encouraged by them at a time when there should be more communication between scientists working in different areas. They urge, as a quick way of getting the message over to publishers, that scientists should ask their libraries to think twice about subscribing to new commercial journals, and themselves refrain from publishing in them. Somewhat idealistically, they talk of the need for an international agency to examine the founding of new journals, and further suggest that national chemical societies (though they be far from perfect and their track records not always shining bright) should set up "an impartial mechanism for evaluating the need for a new journal and require that criteria for assuring the level of quality is met". This committee's concerns would include "a set of criteria for refereeing practice, statistics about rejection rates, criteria for terminating a journal, restrictions on language of national origin of work, page charges, and so on."

The Chemical Society's Director of Publications, Dr L. C. Cross, confirms the complaints of the group and sums up the overall problem of over-specialisation and the haphazard division of new knowledge into packages of narrow interest in the following terms: "This has merely resulted in ever multiplying costs to the consumer and greater profits to the producer. Such results are inevitable because the package is not being altered fundamentally, only the label is being changed, with results that any housewife would predict".

Another group of scientific authors and editors, including Dr Monty Finiston (*Materials Science*), Professor J. P. Hartnett (*International Journal of Heat and Mass Transfer*) Professor N. Kurti (*International Monographs of Solid State Physics*), and Sir Harold Thompson (*Spectrochimica Acta*) lamented over soaring prices in a letter to Sir Walter Coutts, chairman of their publishers, Pergamon Press Ltd. They also proposed a remedy which, a sceptic might remark, was not guaranteed ab-

olutely to reverse the trend.

"We are alarmed" (they said) 'at the regular annual exorbitant price increases on books and journals, not only by Pergamon but also by other publishers. These augmentations have practically eliminated purchases of publications by private individuals. Libraries, with fixed or reduced budgets, are facing impossible choices between urgently needed publications. Also we deplore the fact that resulting from these price increases students are buying fewer and fewer books for their personal use. Here we need the kind of imaginative leadership that Robert Maxwell can provide in order to reverse disastrous pricing trends in the publishing industry and to help meet these other difficulties'.

On the strength of this recommendation (and of rival claims for the merits of a counter proposition) Mr Maxwell was asked by *Nature* whether Pergamon was specially culpable over the issues at stake and what, if anything, he proposed in order to make life more bearable for subscribers.

In the first place, says Mr Maxwell, Pergamon's total of 142 scientific journals represents a growth of less than 5% over the past two years. "There is a growing number of people doing

research in a growing number of subjects and because of the pressure on existing publication machinery it is inevitable that new journals emerge to meet these new demands. These chaps have a point about proliferation but in no case do we, the publishers, initiate a new journal; not a single one. A group of scientists will write in and say they feel there is a need for a journal in a certain area, so we talk about it and carry out a detailed, world-wide survey, and if there is a genuine need then we go on from there. But really, the actual proliferation has slowed down immensely".

Naturally enough, Mr Maxwell defends the standards of the referees used by Pergamon. Like the editors and editorial boards of his journals, the refereeing is "of the highest standard". Sometimes, he adds, a paper rejected by a Pergamon publication will turn up in the journal of a learned society, although this is not to say that in a vast output of 20,000 papers a year there is not "an occasional slip-up if a referee has not been as careful as he ought to have been".

As for the cash angle, Mr Maxwell is in scientific publishing because he likes to be of some help to science and, not having been to university, he does

Dispute halts abstracts scheme

Colin Norman, Washington

A DISPUTE between the American Institute of Physics (AIP) and the London-based Institution of Electrical Engineers (IEE), has brought to a temporary halt an attempt to rationalise the production and marketing of physics abstracts. It seems that the IEE, for financial reasons, has abrogated an agreement worked out between the two organisations last summer, and as a consequence, the AIP has terminated the marketing of IEE information products in the United States—a service which the American society has been performing for the past five years. The disagreement came to light recently when the executive directors of the societies traded public statements on the matter, but both have expressed the hope that a new arrangement can still be worked out.

The cost of producing abstracts of physical articles has shot up in the past few years, with the result that *Physics Abstracts*, the key IEE service, has increased in price from \$12 in 1967 to \$380 in 1974. It is thus circulated chiefly to institutions rather than to individuals. The

AIP abstracting services, however, are geared at least partly to the needs of individual subscribers, and in that respect the two services are complementary and compatible. Moreover, since more than 25% of the IEE abstracts are taken from papers published in the primary journals of the AIP, there is clearly considerable scope for cooperation in the production of the abstracts.

Against that background, officials of the two societies worked out an agreement in June last year under which the AIP would supply the IEE with computer-readable abstracts of articles published in AIP journals. The agreement would also have given the AIP considerable influence over the content and design of IEE abstracts, and the British society would have reimbursed AIP for the service. The arrangement would have permitted multiple use of each computer tape, thereby cutting down production time and costs of abstract journals. IEE officials later felt that they were getting a raw financial deal, and reneged on their commitments, whereupon the AIP discontinued marketing IEE products. IEE abstracts are now marketed in the United States by the Institution of Electrical and Electronics Engineers (IEEE).

the best he can by seeing that the work of scientists is disseminated. The profit motive is not uppermost in his mind, and such profit as accrues is useful chiefly to ensure that the overall operation runs efficiently and as a subsidy for prestige loss-leaders like *Leonardo* (bridging arts and science), *Archives of Oral Biology* (for dentists) and *Marcellia* (The International Journal of Phytopathological Morphogenesis and Cécidology).

Among the numerous examples of subsidised services to science which Mr Maxwell says he can name off-hand is the Russian translating service which, although reduced after its first five years of operation, still produces of the order of 20,000 pages a year. The company's last published report showed a profit in the region of £200,000 a third of which came from books. (Trading profit for the year ended September, 1973, was expected to be £292,000.

As a sign of his good intent, Mr Maxwell instances his plans for *Tetrahedron*, a top organic chemistry journal with some 5,000 subscriptions (mainly at the library rate of £102 a year, for which a library receives something like 12,000 pages of information). As an experiment, *Tetrahedron* is to be circulated in the form of expanded abstracts. Thirty papers in this form will occupy 60 printed pages, whereas the full papers would need 300. The net result is a saving of 75% to 80% in costs to the subscriber, who simply writes for the detailed version of such papers as appeal to him, at no more than the photocopying cost. He also plans to cut subscription costs by developing a new typesetting technique for scientific material by harnessing the computer to the traditional archival type of journal; and when the annual rise of 10% in the volume of published literature makes it impossible for libraries to house any more magazines, he plans to be a leader in the field of microfiche and microfilm reproduction. Yes, he says, the portable microfiche reader will eventually oust the paper magazine. Which is all very fine with McLuhan, no doubt, but not much of an answer to the immediate problems of Alec Henderson, Charles Bubb, Kenneth Humphries and a cast of thousands of their library users.

Soviet journals have their problems too

from our Soviet Correspondent

THE catalogue *Gazety i Zhurnaly SSSR* (Periodicals of the USSR) for 1974 lists some 600 scientific and technological titles (excluding abstract journals), more

than 500 of them in Russian, with Ukrainian—30 titles—as the next most prolific language. According to an article in *Pravda* by Candidate of Technical Sciences R. Ivanov (December 21, 1973) this plethora of specialist information is proving more and more difficult to digest, even on the assumption that a given reader will require to scan only the literature of his own speciality. A survey of 25% of the Soviet specialist journals, Ivanov claims, reveal some significant deficiencies.

Although it has been obligatory since 1967 for all scientific and technical publications to be accompanied by abstracts, only half of the journals studied did include them. Moreover, when abstracts occurred they had frequently not been revised by the editors with their intended readership in mind but were left in the unsatisfactory state in which they came from the authors, who write down as an abstract "Whatever God puts into their souls". Ivanov also criticises the practice of certain journals of printing the abstracts on narrow strips of coloured paper, bound into the front or back of the journal—this may save paper but wastes everyone else's time. Best of all, he maintains, is the practice of printing the abstracts on detachable, fileable cards, which saves "many thousand of man hours". (One assumes that the Soviet reader, consulting the journal in a library, would never be so anti-social as to remove such an abstract to his private collection—or is there some security system to prevent this?)

Other major criticisms are the waste of paper at the end of articles (which could be utilised for announcements of forthcoming books, conferences and so on) and the absence in some 80% of the sampled journals of a submission date indicating the age of the material. Seventy-five per cent of the journals ignored the practice ("a sign of high culture") of putting the title, number and date of issue of the journal on every page—thus causing specialists much delay in hunting through their collections of photocopies.

Faced with this abundance of journals, the increasing use of computer indexing and the physical and logistic problems of storage, Ivanov suggests not only such ideas as the introduction of standardised Union-wide identification symbols, and the printing of the abstract and bibliography together for simultaneous scanning (some of which problems are already under consideration by working groups) but the proposal, possible only in a very centralised system, that a single, unified physical format should be adopted for all scientific and specialist journals published in the Soviet Union for greater convenience in shelving.

NIH leaders attacked and defended

Colin Norman, Washington

THE longstanding dispute between officials at the National Institutes of Health and their superiors in the Nixon Administration has moved on to new battlegrounds—the correspondence columns of the *Washington Post*. The disagreements, which stem chiefly from the fact that the Administration has been attempting for the past year or so to centralise control over biomedical research policies in the Department of Health, Education and Welfare and the Office of Management and Budget, came to a fresh head recently when Dr John Sherman resigned as deputy director of NIH. He cited a number of intangible reasons for his departure, prominent among which was a complete lack of understanding between officials at NIH and administrators in the Department of Health, Education and Welfare (see *Nature*, 247, 172; 1974).

The *Washington Post* then criticised HEW officials in an editorial, a move which stung Dr Charles C. Edwards, Assistant HEW Secretary for Health, into firing off a letter to the newspaper, in turn criticising the "inadequate leadership" at NIH. Not surprisingly, several scientists at NIH drafted a reply to Edwards's letter, circulated it around the NIH campus for a couple of days during which it gathered 460 signatures, and dispatched it to the *Post* last week.

The *Post* editorial had called on the Nixon Administration to give NIH the autonomy that it has had in the past, to restore its "world-wide reputation for excellence". But Edwards argued in his letter that an institution which spends \$2,000 million a year cannot be completely insulated from budgetary and managerial control. He also maintained that confidence in NIH has "diminished in the past through inadequate leadership and a misguided sense of the place of research in the nation's efforts to solve its health problems."

What really stung NIH scientists, however, was a reference in Edwards's letter to the fragmentation which has been taking place at NIH, particularly the increased autonomy that has been given to the National Cancer Institute. "If the NIH leadership had been more receptive and responsive", he argued, "we might not have witnessed the removal of the cancer research effort from the administrative control of NIH, a move that threatens the further dissolution of biomedical research efforts".

What Dr Edwards seems to have forgotten is that when Congress was debating the National Cancer Act, the NIH leadership fought a battle within the Administration to prevent the National

Cancer Institute from being given autonomy. The move was partly successful because the Act was amended at a late stage in the House of Representatives to keep the cancer institute at least partly within the structure of NIH. At that time, however, the Administration had lent its official public support to a bill which would have set up the National Cancer Institute as virtually an independent body.

The NIH scientists, in their reply, state that "with Dr Edwards, we deplore the fragmentation of the NIH, but it is essential to recognize that this fragmentation was opposed by the NIH administration". The nub of their argument about management control of biomedical research is that "we fully appreciate the importance of political and economic considerations in the process by which the nation decides how much is to be spent on biomedical research. But whatever that sum may be, we believe that the nation will reap the largest benefit if scientific and medical considerations govern the programs and administration of the NIH".

So far, thanks partly to the public airing of their grievances and support from influential members of Congress, NIH scientists have been relatively successful in their efforts to maintain at least a semblance of autonomy for the institute from management control by HEW and OMB. In the process, however, the three top officials who ran NIH during the first four years of the Nixon Administration—Robert Q. Marston, NIH Director, Robert Berliner, NIH Associate Director for Science, and Sherman—have either been forced out or have resigned to take less frustrating jobs.

When is a forgery not a forgery?

John Hall

CURIOUSER and curiuser, remarked at least half a dozen scholarly gentlemen who found that they had stepped out of the grim reality of a February night in London and into the topsy-turvy realm of Vinland. Falling through the portals of the Royal Geographical Society they learned on the one hand that the renowned map of Vinland was drawn in twentieth century ink, and on the other that this did not necessarily mean that it wasn't a genuine work of fifteenth century scholarship illustrating a Norse voyage of discovery to North America.

Turning aside from the richest collection of medieval documents in the world, George Painter made his way from the British Museum's research department to Kensington Gore in order to explain that there was no mistaking the work-

ings of a *bona fide* medieval mind in the cartography, the linguistics and the scholarship of the map which is now the property of Yale University. ("O.K. here's your hundred thousand to buy the thing", Andrew Mellon is reputed to have said, "but if it turns out a forgery, well just don't come calling for anything ever again". Right now he must feel justified in stamping his foot.)

Meanwhile, looking up from his micro-analytical mass spectrometer, burly, close-cropped Walter McCrone, of McCrone Associates, Chicago, delivered himself of the opinion that the likelihood of a pigment of the crystalline size and shape of that found in the Vinland Map ink being used in a map of AD 1400 'could be compared with the likelihood that Admiral Nelson's flagship at Trafalgar was a hovercraft.'

Only time will tell, held Painter, how we might arrive at a scenario admitting that a fifteenth century manuscript might remain genuine in spite of its being drawn in an anatase pigment containing titanium dioxide particles first manufactured only fifty years ago. Leaving aside time travel, the prospects for such a scenario seem to fall into three categories. First, suppose somebody happened on the Vinland Map, thought its outline had become too faint for comfortable scanning, and dashed over it with modern ink. Sorry, says McCrone, but there's no sign of further pigmentation below the titanium dioxide ink.

Suppose then that an ingenious scribe, scraping about for a new line in inks, decided to use the very compound which wasn't used again for that purpose until about 1920? No way, says McCrone. Naturally-occurring titanium would contain impurities such as iron and manganese: what's more, the titanium dioxide used was a precipitate, the shape and size of whose particles were indistinguishable from those used in anatase.

Allright, says Painter. Textual evidence indicates that the version we have is a copy of earlier versions: is it not possible then that the present version is simply one more copy, a modern one, made from a genuine version of the map, if at several removes? Not even the implacable McCrone can gainsay this possibility: it is not within the realms of spectrometry. Without any sort of material support, the thesis doesn't look all that powerful, but Painter points out that the chemical analysis of the ink is simply one piece of evidence, which has to be weighed alongside considerations such as the quality of the scholarship and the thinking embodied in the map. These aspects are not quantifiable, and may seem less tangible than a scientific analysis, but they nevertheless add up to evidence, and deserve to be weighed equally with McCrone's findings.

Painter's hopes for a future recon-

ciliation of the chemical evidence and the scholarly hunch is heartwarming, but it must be reported that his is a voice crying in the wilderness, and the weight of opinion seems to be that the jig is up for the map's supporters. Another of the British Museum's research team, Mr A. D. Baynes-Cope, has had his doubts for some years, and told Yale as much when they asked in 1968. The thing has been scrubbed, as if in attempt to give it a weathered look, and the worm holes in the vellum have been patched in an amateurish way, says Baynes-Cope. The British Museum's own craftsmen could have made a better forgery.

Scholars being what they are, nobody is prepared to say exactly who was the Vinland Map's Mr Big. The name of a European professor of canon law, one Jelic, is bandied about, but as he died in 1922, that wouldn't have left him all that much time to do the job after the introduction of anatase inks. One thing's for sure, and that is that the forger (assuming the thing was forged) didn't make a fortune out of his work. The map was sold for only 3,500 dollars as recently as the late fifties, and even McCrone is bound to admit that the effort was worth considerably more than that. His theory is that maybe some gifted academic drew the map for his private amusement, and that it only assumed importance, and cash value, when it was discovered after his death.

Either way, no real harm has been done anywhere outside the Mellon Foundation's cheque book (if they were the anonymous donors. If not, *pace* Andrew Mellon). Another of the British Museum's sceptics, Dr Helen Wallis, points out that at best the map would have been significant evidence that the Vikings had a good working knowledge of the North American coastline fifty years before Columbus set sail. At worst it amounts to an interesting forgery which, incidentally, has prompted a work of scholarship (*The Vinland Map and the Tartar Relation*, by Skelton, Marston and Painter) which has turned out to be the standard work in that area of study, regardless of the map's worth.

Health risk in PVC manufacture?

THE British Factory Inspectorate has begun discussions with industry to decide what action may be necessary to protect workers from a possible health risk arising from the use of vinyl chloride monomer (an intermediate used in the manufacture of PVC). The talks follow news that an American PVC manufacturer investigating causes of death

among its employees discovered four cases of rare liver cancer which may have been linked to the use of the monomer. The US Department of Labor has arranged a hearing to examine the extent of workers' exposure to vinyl chloride monomer.

The American deaths were at the works of B. V. Goodrich, and in England the death of a 71 year-old former process worker at ICI is being investigated in order to establish whether or not it was caused by long exposure to the fumes of the monomer. ICI employs 1,200 workers in PVC production, and about 380 of these have been warned of possible risks in their particular work.

Vinyl chloride monomer was first used in plastics manufacture in Germany in the 1930s, and in the early days of its use in Britain and the United States a threshold limit value (TLV) of 500 parts per million was observed because of the fire and explosion hazards involved. However, an Italian study in 1971 showed that rats exposed to vapour with a concentration of the order of 3,000 parts per million tended to develop cancer, and a current TLV of 200 parts per million is observed in industry.

The TUC's medical adviser, Dr Robert Murray, has been visiting plants throughout the country in order to consult with producers and advise workers. The difficulty in arriving at a safe level of exposure to the monomer lies in the fact that a carcinogen risk cannot be quantified as rapidly as a fire or a toxicity hazard, says Dr Murray. Furthermore, the organ most likely to be affected by the monomer is the liver, whose condition is not easily diagnosed by conventional methods of examination.

The immediate priority is to attempt to achieve a really low TLV (say, of 50 p.p.m.), to seek mechanical alternatives to the autoclave swabbing which is the main source of contact, and to make sure that nobody enters an autoclave without air-line apparatus.

"What you've got to say to the workers", says Dr Murray, "is that the risk is small: that apart from the risks involved when he drives his car, and eats too much, and drinks too much, here is another risk which he has got to live with. The production of PVC is too big an operation to be stopped entirely because of this hazard, so what we must do is reduce exposure as far as possible while pressing ahead with the epidemiology".

In Washington, Tony Mazzocchi, Legislative Director of the Oil, Chemical and Atomic Workers Union, said that the union is now in the process of identifying those of its members who have been exposed to vinyl chloride monomer, and that they will be examined for signs of liver damage. He said that there is

at present no basis for the statement that there is little risk from exposure to the chemical, and predicted that in the United States at least, the matter is likely to lead to a long battle. The Oil, Chemical and Atomic Workers Union has recently held a long strike against the Shell Oil Company in an attempt to secure a strong health and safety clause in work contracts.

DMSO cut down to size

Miranda Robertson, Chicago

A CONFERENCE held recently in New York to discuss recent investigations with dimethyl sulphoxide (DMSO) may herald the beginning of the end of the continuing story of this controversial drug. A lipid solvent exploited commercially in household cleaners, it enjoyed a burst of popularity in the early 1960s as a miracle drug active against almost everything from arthritic pain to mental retardation until it was banned by the Food and Drug Administration (FDA) in 1965 on the basis of toxicity tests on animals. Now once again it is the subject of clinical investigation but this time on a more sober note.

In spite of a report by the National Academy of Sciences (NAS) last year, which essentially endorsed the decision made by the FDA in 1965, thirteen laboratories now have authorisation from the FDA to look into the effects of the drug on, for example, scroderma, strains and sprains, and bursitis, in man.

For although the NAS committee, set up in 1972 at the request of the FDA after a public outcry, concluded that profound scepticism was justified, it found the criteria for testing the drug on humans unduly rigorous. Nor is the evidence for its toxicity so terribly alarming. When applied to the human skin (which it penetrates with remarkable rapidity and ease), it causes irritation and blistering in about 50% of cases. In animals, it produces reversible changes in the lens of the eye after the administration of quite unrealistically large doses.

Toxicity apart, there are two main reasons for the bad odour into which the erstwhile wonder drug fell with the authorities. Nobody to this day has the slightest idea how DMSO produces any one of the diverse pharmacological effects claimed for it, for one. For another, it literally gives off a bad odour, within minutes of administration, which not only seems likely to enhance any placebo effect on the patient but makes normal control procedures, such as double blind trials, extremely difficult. Since many of the

claims rest on small but significant statistical differences between treated and control (or differently treated) groups, this constitutes a serious criticism—especially in the absence of any rationale which might be provided by an understanding of the mechanism of action of the drug.

In the view of the NAS committee, however, none of this is adequate reason for refusing investigation on man where controlled animal experiments show that there is a possibility of relieving incapacitating pain or saving life. The latter possibility seems to arise in the treatment of neurological injuries, an area which has not yet been given investigatory clearance by the FDA.

Work on a monkey model at the University of Chicago suggests that DMSO may be more effective than urea (the current drug of choice) in relieving compression on the brain due to subdural haematoma, a common and often fatal sequel of head injuries from any cause. The improvement, according to Dr J. de la Torre, is measured not only in somewhat reduced mortality but in decreased neurological damage to the survivors. There is some evidence in this case that the effects of the drug depend partly on a diuretic action and partly on a more mysterious influence on oxygenation.

How the treatment, which has been extended in animal tests to impact injury of the spinal cord and experimental 'stroke', would emerge from the exigencies of a real neurological emergency remains to be seen. Nor is it yet clear whether DMSO will eventually find a useful and respectable place in the pharmacist's therapeutic repertoire; but it seems likely that with its emergence into the cold light of properly controlled investigation, its days as a panacea are over.

Britain backs seafloor search

To encourage British participation in deep sea mining for manganese nodules, the British Government has offered Rio Tinto Zinc and Consolidated Gold Fields Ltd up to £830,000 towards their participation in an international consortium examining the floor of the Pacific Ocean. The money will be repaid if the project leads to commercial production, and the companies have also agreed that British consumers will have first option to purchase a proportion of any metals produced. The project, therefore, is seen as a means of allowing British companies to break into a new technology, and also in the long term as a possible new source of metals (like copper, nickel and cobalt) for British consumers.

The consortium consists of the Kennecott Copper Corporation (United States), RTZ and consolidated Gold Fields, Mitsubishi Corporation (Japan) and Noranda Mines Ltd (Canada). Its programme will include work on the nature and location of deposits of manganese nodules under the Pacific Ocean

and investigation of methods for working, transporting and extracting useful metals from them. The programme is expected to last five years and to cost more than \$50 million.

Manganese nodules occur naturally on the beds of all oceans, generally at depths greater than 1,000m: the richest

metalbearing nodules have been found in the Pacific at depths of about 5,000m. The content of the nodules varies greatly but the usual range for deep sea nodules is manganese (12% to 34%), copper (0.1% to 1.5%), cobalt (0.1% to 0.3%) and iron (1.6% to 18%).

correspondence

Embryos and Ethics

SIR,—According to Dr Anne McLaren's review (*Nature*, 247: 121) of "The Mammalian Fetus *in vitro*", Professor R. A. McCance "regrets that the Advisory Group recommended that foetuses of more than 20 weeks' gestation should be considered viable, since he feels that this creates a 'shadowy period of 8 weeks' during which abortions can be performed, yet the use of the abortuses for experiments may become illegal".

But the *Infant Life (Preservation) Act 1929* defines destroying any viable fetus as the crime of 'Child Destruction', unless done "in good faith for the purpose only of preserving the life of the mother". It continues "... evidence that a woman had at any material time been pregnant for a period of 28 weeks or more shall be *prima facie* proof that she was at that time pregnant of a child capable of being born alive". But it nowhere says that before 28 weeks the child can be presumed not to be viable, and nowadays a healthy baby of 24 weeks may well live under intensive care.

So if there are any prospects of viability at all a pregnancy may only be terminated to preserve the mother's life, and everything possible must still be done to save the child after delivery. A doctor who fails in this duty, by allowing experimentation or otherwise, would at the very least face a charge of manslaughter by neglect, if at autopsy the baby was found to have been capable of surviving, given proper care.

There have been a few reports of survival from well under 24 weeks, though the real reason for setting a limit at 20 weeks is to allow for mistakes over dates and so on. But, in spite of a wide spread belief to the contrary, the 28th week has no special significance, and anyone experimenting with an abortus at any age near to viability

does so at his peril. Viability is a biological fact, not a matter merely of legal definition, and there can be no 'shadowy period' during which the child may be presumed inviable by reference simply to dates, either for abortion or for any other purpose.

Yours faithfully,

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Adolescents and science

SIR,—I feel that an important aspect may be missing in the interesting research made by A. Ahlgren and J. H. Walberg (*Nature*, 245, 187; 1973).

The attitude towards science among adolescents could have changed for the worse because they may have been taught in a way that gives them the impression that science cannot be understood.

Physics has become so abstract that it is very difficult to teach it so that it remains comprehensible for children and yet up to date in its fundamental principles. For instance, it is very difficult to teach relativity at an elementary level and it is practically impossible to do the same with quantum mechanics. Therefore one either teaches classical mechanics and later says that this is only a first approximation, or tries to begin with relativistic quantum mechanics. The latter is evidently impossible in the present state of affairs. The former easily gives the child the feeling that he is being cheated or at least that the real thing is utterly beyond comprehension. A really epoch making book on 'Modern Elementary Physics' is badly needed—but who is going to write it?

Chemistry is even worse off, especially in the United States and in countries

like Israel where we are copying what they do. It is very often taught in the wrong order. Instead of making it plausible why we think matter is composed of atoms and molecules and why we think they are as large as they are, teachers begin with electrons, nuclei, neutrons without even trying to make their existence plausible. The result is a hopeless mess. Less intelligent children just repeat the story like parrots while the more intelligent ones despair. Then chemistry is built on these shaky foundations with a not much more stable superstructure. England is much better off in this respect than many countries. A professor at the Technical University in Stockholm told me also that the decreasing number of chemistry students is probably due to this way of teaching in high schools.

Biology is often ruined by introducing biochemical concepts not necessary at that level, and which quite certainly cannot even be understood. Presenting the formulae of sugars and amino acids before the elements of chemistry have been taught in the chemistry course is evidently absurd. A very intelligent 17 year old boy in New York told me that he has to write a paper on the chemical foundations of genetics, with nucleic acids and so on involved, without even an elementary knowledge of organic chemistry. The Krebs cycle is also one of the preferred subjects. Any half-way intelligent child feels that he is being bluffed and suspects something wrong in science at large.

We shall have to stop bluffing children and build elementary science courses on solid, understandable foundations if we want intelligent adolescents to turn their preference towards science again.

Yours faithfully,

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news and views

Meteorites which 'bounce' off the Earth

BRIGHT fireballs shooting across the sky are often reported in the press. Meteorite collectors become very excited by these reports because careful analysis of the sightings enables them to calculate the trajectory of the incoming particle and to predict the impact point. The brilliant meteor observed over the western United States and Canada in the early afternoon of August 10, 1972 (see page 449 of this issue of *Nature*) however, would have disappointed them because it did not fall to ground but, in fact, 'bounced' off the Earth's atmosphere and flew off into space again. But it did not disappoint Rawcliffe of the Aerospace Corporation, Los Angeles or Barthy, Li, Gordon and Carta of Aerojet ElectroSystems Corporation, Azusa who fortunately had a satellite-borne near-infrared radiometer in orbit over the United States at that time and detected the radiation from the meteorite. The meteorite path was found to have a perigee of 58 km, and, by assuming that all the loss in kinetic energy was transferred to thermal radiation from the meteorite, these workers found its mass to be 10^9 g. Assuming it to have the density of iron, its equivalent diameter turns out to be 4 m.

From the ground (see *Sky Telesc.*, **44**, 269; 1972) it was seen as a blazing ball of fire, a metallic bluish-white in colour (like a carbon arc or welders torch) moving slowly (~ 15 km s⁻¹) across the sky, leaving behind a train which persisted for about an hour and looked rather like a smoke trail. The brightness of the fireball was intermediate between that of the Sun and the full Moon, the apparent magnitude being roughly -18.

Fortunately meteorites of mass 10^9 g are uncommon—recent flux estimates indicate that one should hit the Earth every 10 to 20 yr. If this meteorite had hit the ground it would have produced a crater with a diameter of about 200–300 m. The failure of this meteorite to impact, however, is a much rarer occurrence and can only happen to the larger meteoroids (mass greater than a few grams). The 'bouncing' meteoroids enter the atmosphere almost tangentially and an estimate can be made of their frequency by comparing the cross-sectional area of the Earth plus its atmosphere with the area of an annulus of thickness one scale height (~ 7 km) surrounding the Earth at the height of the meteor region (100 km). This indicates that only 0.2% of the meteoroid flux will bounce, the remaining 98.8% falling to ground.

Rawcliffe *et al.* are not the only scientists to have recorded a 'bouncing' meteorite. On November 11, 1968 a camera mounted on the payload of a 'Hibal' balloon at 31 km above Queensland, Australia photographed a tangential meteor trail. Bigg and Thompson (*Nature*, **222**, 157; 1969) concluded that this meteorite consisted of four sizeable pieces of solid material together with a vast number of tiny dust particles.

It is thought that the effects of grazing meteorites have been observed by the micrometeoroid detector on the HEOS 2 Earth orbiting satellite. Hoffmann, Fechtig, Grün and Kissel (COSPAR, Konstanz 1973) report that this satellite from time to time encountered localised dense swarms of micrometeorites. These swarms were produced, according to Kaiser (University of Sheffield), by the fragmentation of grazing meteorites.

Returning to the report by Rawcliffe *et al.* it would be interesting to see estimates of the amount of energy lost by the meteorite due to frictional drag in the atmosphere. At 58 km, the meteorite perigee, the mean free path is about 0.02 cm and a shock wave could possibly have been produced. If this had occurred, the meteorite would have lost energy to atmospheric turbulence and the authors estimate of its size would have to be reduced. Only one of the ground reports recorded any sound. This was from an observer in British Columbia, where the meteorite was leaving the atmosphere, who reported a faint rumbling 2 to 3 minutes after it had disappeared.

D.W.H.

Light and plant disease

THE effect of light on the predisposition to, and subsequent manifestation of, virus diseases in plants has been studied for many years. Bawden and Roberts (*Ann. appl. Biol.*, **34**, 286; 1948) found that plants grown for long periods at low light intensity or subjected to periods of darkness before inoculation were more predisposed to virus infection and often produced more primary local lesions and/or became systemically infected, and thus diseased, in a shorter time.

Virus-infected plants often show more severe systemic diseases when grown in poor light, though subsequent work has shown that many viruses multiply more rapidly in plants that are grown at their optimum light intensity and photoperiod. Shorter photoperiods immediately after inoculation can reduce or delay local lesion formation, whereas high humidity often enhances the development of symptoms in infected plants (Yarwood and Fulton, *Methods in Virology*, **1**, 237; 1967).

Kirkham, Hignett and Ormerod reported recently (*Nature*, **247**, 158; 1974) that the response of cucumber plants to the inoculation of a virus was reduced by interrupting daylight with intermittent 2 min periods of darkness. The authors suggest that the reduction in the formation of local lesions on the inoculated plant was the result of a change in the hormonal complex within the plants. This change could have been stimulated by the intermittent dark periods, especially as the total time of light and dark were equal in all experiments so that the amount of photosynthate was equal in all plants.

Kirkham *et al.* did not, however, study the numbers of virus particles colonising the plants and multiplying within them. Nor was any observation made later to record total numbers of local lesions formed. Thus there is no information on whether interrupting daylight has an effect on initial colonisation of the plant by the virus or whether formation of local lesions was merely delayed and not inhibited. The effect of intermittent lighting on the multiplication and translocation of the virus or the subsequent systemic disease that this virus induces in cucumber was not mentioned.

The East Malling workers studied one virus in one plant and related the plant responses to those induced by two fungal pathogens in other plants growing in conditions of intermittent light. The hypothesis that plant hormones, stimulated by the intermittent light, influence plant disease responses does not take into account the many other physiological systems within plants that are photosensitive and

related to disease response. Light intensity and photoperiod can influence cytochrome and enzymatic systems as well as many other biochemical and metabolic activities. The proposed hypothesis would have more credence if the experiments described had been markedly reversed or altered by the addition of plant hormones.

It is also well known that many bacteria and fungi are themselves photosensitive so that changes in photoperiod and light intensity may have a pronounced effect on their pathogenicity as well as the plants' response. Thus it is premature to equate fungal pathogenicity or the severity of a fungal disease with a hormonal response in the plant induced by intermittent light.

It seems likely, however, that further studies along these lines may provide a better understanding of the complex of plant and pathogen and disease induction. Research workers studying the development of diseases or the assay of fungicides should now be acutely aware of the importance of incident light as well as the other environmental factors such as temperature and humidity, in order to obtain reproducible results.

From a Correspondent

New theory of evoked responses

THERE is a kind of computational equivalent of Parkinson's law, that computation expands to fill the space available. Putting it another way the computer seems to be seen by some as a helpful amplifier of the seriousness and scientific value of research; and at best as a guarantee that the frontiers of knowledge really are being rolled back. In this context it is hardly surprising that the computer has become one of the most significant, and indeed symbolic, denizens of laboratories undertaking research in neurophysiology and psychology. The suspicious might wonder whether the uses of the computer in its symbolic role are quite as well thought out as they ought to be.

The particular function that computers usually play in neurophysiological and psychological research is that of doing statistics on various biological signals, a typical instance being the averaging of electroencephalogram (EEG) activity following the application of stimuli. The argument is that the shape of the electrical response to the stimulus is not discernible in the single recorded trace, because it is buried in uncontrollable spontaneous activity which irritatingly obscures the actual response to the stimulus, but at the same time also obligingly provides the requirement for a computer. By recording the responses to several dozen stimuli, digitising and summing the resultant waveforms in a computer, the consistent features of a response will add together. As the sum of these consistent features grows larger, the inconsistent fluctuations tend to sum to zero, and the signal stands out dramatically from the noise. The procedure seems both logically straightforward, and with the advent of small computers, even indispensable for serious research on the nature of the electrical responses of the brain to stimulus events. Sayers, Beagley and Henshall who are perhaps better versed than many on analysis of biological signals analysis have, on page 481 of this issue of *Nature*, gone directly, if somewhat iconoclastically to the heart of this matter by quite rightly questioning the apparently unassailable logic of this, by now standard, procedure.

Sayers *et al.* point out that underlying the averaging technique is the idea that the electrical response to a stimulus is added to the background of spontaneous activity, so that summing the responses actually selects out this additive component. But this, they declare, is an unexamined assumption,

Evolution in action

IF current views on evolution are correct, it must be the case that on occasions a gene changes so as to produce an enzyme with an altered function. It is therefore a challenge to experimentalists to produce such a change by selection in the laboratory. The obvious approach to this problem would be to keep a bacterial population capable of using substrate *S*, and also capable of using a related substance *S'* but with low efficiency, on the latter substrate, and at the same time to expose the population to a mutagenic agent. Bacterial populations do adapt to such circumstances, but they usually do so by producing enormous quantities of the original inefficient enzyme rather than by evolving a better one. Typically, a 'constitutive' mutation takes place in the system regulating the activity of the gene, so that the gene is permanently switched on; in this state, up to 20% of the bacterial protein may consist of this one enzyme.

Betz, Brown, Smyth and Clarke (*Nature*, **247**, 261; 1974) recently summarised some experiments in which an understanding of the mechanisms of gene regulation have made it possible to bring about in the laboratory the evolution of a new enzyme specificity. The bacterium was *Pseudomonas aeruginosa*, and the substrates were amides used as a source of nitrogen. Normal strains can use acetamide and propionamide; their enzyme acts with very low efficiency on the 4-carbon compound butyramide, but the bacteria cannot utilise this substrate, because butyramide does not induce the production of the enzyme, and in fact acts as a competitive inhibitor of its production. The enzyme is inactive on the 5-carbon compound valeramide.

Betz *et al.* obtained several new strains, including some capable of utilising valeramide, by way of two intermediate steps. The first step was a constitutive strain C11, obtained by selection on formamide—a substrate used with low efficiency by the species. Strain C11 cannot grow on butyramide, because the gene producing the enzyme is repressed by this substrate. From strain C11 a mutant B6 was obtained with a much higher activity on butyramide, but still retaining its activity on acetamide. A third mutational step, starting from B6, gave rise to a class of mutants able to utilise valeramide for growth. A number of other enzymes were obtained by this and other routes, including enzymes active on phenylacetamide.

For biochemists, the existence of a group of very similar enzymes with different specificities may be a useful tool in studying mechanisms of enzymation. For evolutionists, it is both important and reassuring to know that a new enzyme specificity can be produced by very few mutational steps. It is also interesting that each step could in certain circumstances be selectively advantageous, so that there is no need to postulate a long 'random walk' before a new function is achieved.

From a Correspondent

and upon examination, it turns out, a mistaken one. They conclude that what the stimulus probably does is to reorganise already existing components of the spontaneous EEG pattern, and not provoke a new electrical event superimposed upon it.

They cast the problem into the Fourier domain. The

Fourier theorem states that a signal which can be described as varying with time can be alternatively described, without loss of information, as the sum of a set of sinusoidal waves, of specified frequencies, amplitudes and phases. (Phase can be thought of as the time, or relative time, of occurrence of some conspicuous point, for example, the maximum in these waves.) Thus frequency analysis of a signal consists of discovering which sinusoidal frequencies exist in the signal and what amplitudes and phases each of these various sine waves has.

What Sayers *et al.* have shown is that if the supposed response to a stimulus (in their case an auditory one) is compared with a similar length of EEG before the stimulus, then the average power (the square of the amplitude) of the frequency components in the response is not actually any different from the average power in a pre-stimulus interval. If the response were an event superimposed on the spontaneous activity, it would clearly add amplitude (and hence power) in the spectrum. That it does not (and the experimenters display a comparison between the amplitudes of the ten principal frequency components of EEG following supra-threshold and sub-threshold stimuli), seems to indicate that the stimulus must reorganise the phases of pre-existing frequency components of the EEG. This has the effect of making an averaged response apparently stand out from a background, but for a quite different reason than that usually assumed.

In the phase data, the phases of the ten principal sine wave components of the total signal in supra-threshold responses were found to be closely specified with respect to one another and the specification was consistent over a number of runs. In the EEG following sub-threshold stimuli on the other hand, the starting points of all the various component waves measured in different runs were scattered uniformly and apparently randomly over the whole range. Thus a stimulus did seem to have the effect of imposing a specific organisation on the phases of existing frequencies.

To test their conclusion, Sayers *et al.* used the reconstitution technique suggested by the Fourier theorem. They found the amplitudes of the frequency components in sections of spontaneous EEG activity (with no stimuli) from their subjects. Then they ordered the starting points of synthesised sine waves at the different frequencies according to the discovered phase relationships of these frequencies in the post-stimulus signal. The synthetic signals thus produced bore a strong resemblance to 'real' responses, and indeed supported a high coefficient of correlation with them. Reconstituting the other

way round, that is to say by finding the phase relationships in a length of spontaneous EEG and then using these to specify the starting points of a set of sinewaves whose amplitudes were found from an actual response, produced a pattern quite different from the post-stimulus records: and one which had virtually zero correlation with them.

This conclusion is the opposite of that derived from the assumption underlying the conventional use of post-stimulus time averaging. It suggests a much more appropriate way of analysing EEG responses, as well as a quite new concept of how they arise. For those researchers who use the evoked response of the EEG simply as an indication that following a stimulus something does actually happen inside the subject's head, this result will probably be of not much significance. For those whose study of evoked responses is any more searching, however, this is a result that can hardly be ignored, even if it does require confirmation in circumstances different than those described by Sayers *et al.*

from our Experimental
Psychology Correspondent

Heliocentric theory in China

from a Correspondent

By the end of 1973 most historians of astronomy had had their fill of the life and work of Copernicus. A report of a quinqucentenary celebration meeting held in China last June, however, does add a new slant to the innumerable papers already published on the topic (*Scient. Sin.*, 16, 364; 1973).

It begins in classical style with a quotation from the works of Chairman Mao: "What is correct invariably develops in the course of struggle with what is wrong. The true, the good and the beautiful always exist by contrast with the false, the evil and the ugly, and grow in struggle with the latter . . . Copernicus' theory of the solar system and Darwin's theory of evolution were once dismissed as erroneous and had to win through over bitter opposition." This sets the scene for a discussion of the struggle for acceptance of the heliocentric theory in China.

The initial step is to show that ideas of the Earth's motion existed in China long before the birth of Copernicus. It is eventually admitted that most of the passages offered in proof of this contention must actually be construed as referring to rotation of the Earth, rather than to its revolution round the Sun. (One or two of the quoted extracts require the eye of faith even to discern terrestrial rotation.) These passages, however, enable the authors to claim that the two concepts of a stationary

Earth and an Earth in motion had co-existed since ancient times, as had the struggle between them. This struggle was so prolonged because, "until production, commerce, geographical discovery and astronomical observation developed to a new stage, and the new class force in society emerged on the political arena, a scientific heliocentric theory could not have been fully verified".

With the advent of the Jesuits in China, heliocentric views arrived from Europe, but only in a veiled form since the Roman Catholic church was opposed to the concept. "Since", however, "they intended to use science and technology as a means to win the confidence of the Chinese, the Jesuits had to make their transmission of scientific knowledge through the test by scientific practice. In the field of astronomy, they must make their forecast of celestial phenomena tally with actuality. Thus, the objective laws of nature compelled them to deviate from their usual belief and seek assistance from the works of Copernicus, Galileo and Kepler."

In the early eighteenth century, the arrival of instruments employing the concept of a heliocentric system—two armillary spheres from England are specially mentioned—forced the Jesuits on the defensive. But knowledge of the existence of these spheres, and of other relevant material, was kept within a limited circle. The crucial date for a more general dissemination of the heliocentric theory in China only came in 1859. (The significance of this date for European science is not mentioned.) In that year, the first Chinese translation of a modern astronomy text—Sir John Herschel's *Outlines of Astronomy*—made its appearance. By the end of the nineteenth century, the Copernican hypothesis had become sufficiently well known in China to be referred to in popular ballads.

Clearly, the history of the heliocentric theory holds great attraction for the Chinese as an exemplar of the ideology of struggle. But this Copernicus meeting has more significance than that: it provides a further indication that China is gradually moving back into the world scientific community. More still, it is admitted that China has long lagged behind the Western world in science, and still does so today; that this deficiency is something that must be remedied. The final words of the report are worthy of note in this connection. "China has fallen behind a great length in science . . . to compare with advanced world level, there is still a wide gap. We are still a developing country. Steps have just been taken to the study of the afore-mentioned significant theoretical problems of science. In the spirit of 'Making the past serve

the present and foreign things serve China', we will learn from the people of all countries. Like Copernicus, we will have the courage to break paths where none have gone before and to scale heights yet unclimbed. We will strive to catch up with and surpass the advanced world level in the near future to make a greater contribution to humanity."

Paradox of Earth's core resolved

from our *Geomagnetism Correspondent*

ALTHOUGH convection in the Earth's mantle is now widely accepted among geophysicists as a strong working hypothesis, it took a long time for this happy state of affairs to be reached from the original proposal of the concept. By contrast, the credibility of convection in the outer core has always seemed much greater and less likely to be eroded by expressions of doubt. To a large extent this is because the fluidity of the outer core was demonstrated by seismology many decades ago, whereas the acceptance of mantle convection requires belief in the more difficult and recent concept of plastic or semi-fluid flow in a solid or near-solid. But core convection also gained credibility by the necessity of explaining the origin of the geomagnetic field. The need for mantle convection depends on the acceptance of continental drift and sea-floor spreading, acceptance which was gained only with a struggle and is even now not quite universal. The existence of the Earth's magnetic field, on the other hand, has never been in doubt, and an origin other than core convection has never proved viable.

Of course, core convection is still an article of faith rather than a proven phenomenon because the existence of fluid, rotating or not, does not in itself imply the presence of convection. Even so, geomagneticians have been somewhat dismayed recently to find Higgins and Kennedy (*J. geophys. Res.*, **76**, 1870; 1971) and Kennedy and Higgins (*J. geophys. Res.*, **78**, 900; 1973) purporting to show that the core is stable against thermal convection. For convection to occur in the core, the adiabatic temperature gradient throughout must be less than the melting temperature gradient, for if this is not the case the required instability cannot develop. Moreover, neither can there be convection if the actual temperature is above the melting temperature and the actual temperature gradient is less than the adiabatic gradient, for convection is then less effective than conduction as a heat transfer mechanism. That the actual temperature throughout the outer core is indeed at or above the melting tem-

perature can hardly be in doubt because the outer core is fluid. It is thus usual to assume that the actual temperature is higher than the melting temperature everywhere except at the inner core-outer core boundary where the temperatures are equal, supposing the boundary to represent the solid-liquid phase change of the core material.

If convection occurs, and because it is a very efficient heat transfer process, the actual temperature at all points will be very close to the adiabatic temperature—and the situation generally assumed hitherto is that both of these curves lie above the melting point curve. Higgins and Kennedy, on the other hand, revised the melting temperature of pure iron (the supposed principal core constituent) and the adiabatic temperature throughout the core, and concluded that the adiabatic temperature curve lies below the melting point curve. The core is still fluid, of course, and so the actual temperature curve is still above the melting temperature curve. But now there is no convection, and the actual and adiabatic temperature curves lie on opposite sides of the melting point curve.

It would be wrong to suggest that geomagneticians have been seriously concerned about all this; so sure are they of the reality of core convection that they have generally been content to sit back and wait for someone to point out the flaw in the Higgins-Kennedy argument. Their wish has now apparently been granted by Frazer (*Geophys. J.*, **34**, 193; 1973). As Frazer points out, Higgins and Kennedy are on shaky ground right from the start because their melting curve is for pure iron, whereas the core almost certainly contains a proportion of lighter elements. The precise effects of these lighter constituents are not known except that they are certain to decrease the melting temperature; but in any case Birch (*Geophys. J.*, **29**, 373; 1972) has concluded that in the present state of knowledge the melting temperature of iron at core pressures cannot be given with an accuracy greater than ± 500 K.

Higgins and Kennedy are thus less secure than they might seem because of the old and common problem of uncertainty in core parameters. But Frazer goes much further in claiming that the Higgins-Kennedy argument is not valid even if their melting temperature curve is accepted because the assumptions made in their calculation of the adiabatic gradient are demonstrably inappropriate to the core; for example, one of the two methods used by Higgins and Kennedy involved equations derived by Valle (*Annali Geofis.*, **5**, 41; 1952). Strictly, these equations apply to a solid, but Valle supposed that at very high pressures a liquid behaves sufficiently like a solid for the same theory to be ap-

plicable. At the same time, however, he supposed that the core behaves like a liquid in that the shear wave velocity is zero. The assumptions are thus physically incompatible—a defect which, Frazer claims, invalidates the theory's application to the core unless some more convincing justification can be given. Similar claims are made against the second method (involving Gruneisen's parameter Γ) used by Higgins and Kennedy.

But if Frazer's refutation of the case against convection put forward by Higgins and Kennedy is valid, it cannot necessarily be taken that convection actually occurs. Even if the simplifying assumptions adopted by Higgins and Kennedy are avoided, integration of the more general equation for the adiabatic gradient is still critically dependent upon obtaining accurate values of the core's coefficient of thermal expansion (α) and specific heat at constant pressure (C_p). In fact, accurate values of these parameters are not known. It turns out that comparatively small changes in the α/C_p ratio which are well within the present limits of uncertainty are sufficient to place the adiabatic temperature curve well above or well below the melting temperature curve. According to Frazer, it is thus not possible to say whether convection in the core is feasible or not. To that extent the convection implied by the existence of the geomagnetic field remains but an article of faith.

Heterogeneous nuclear and messenger RNA

from our
Molecular Genetics Correspondent

ONE of the most important characteristics of messenger RNA synthesis in eukaryotic cells is the processing system in the nucleus which cleaves the mature messenger molecule from its much larger precursor. Because only a small proportion of the heterogeneous nuclear RNA (HnRNA) comprising the precursor is utilised to produce messengers—most of it is rapidly degraded within the nucleus—the maturation process represents a critical step in gene expression in eukaryotes. Three articles in the first issue of *Cell* are devoted to intriguing questions raised by the relation of HnRNA and mRNA.

A vital step in processing the large HnRNA to the much (up to ten times) smaller mRNA is the addition of a sequence of polyadenylic acid, probably one base at a time, to the 3' end of the HnRNA molecule. This is then cleaved to generate the poly(A)-containing mRNA. In eukaryotic nuclei, the length of this poly(A) is usually about 200 bases and inhibition of its addition prevents messenger production.

What is the function of the poly(A) sequence? Is it related solely to the maturation process, perhaps because it is needed for nucleocytoplasmic transport of mRNA, or does it serve some function in the cytoplasm? That its role is not solely nuclear has been inferred from experiments which demonstrated the presence of poly(A) in viral RNAs which function solely in the cytoplasm. Support for the view that poly(A) plays some part essential to the function of all mRNAs in eukaryotic cells is provided also by the observation that the messengers of mitochondria possess a short length of poly(A).

Mitochondria possess a protein synthetic system which differs in several ways from that of the surrounding cytoplasm, in some ways showing analogies to bacterial protein synthesis. The presence of poly(A) sequences has been used to isolate a mitochondrial RNA fraction which seems to represent the messengers; their length of poly(A) is fifty-six bases, somewhat shorter than

that of cytoplasmic mRNA. An observation now reported by Hirsch *et al.* (*Cell*, **1**, 31; 1974) is that the size of the poly(A) fragment is virtually identical in human and insect mitochondria. Although no function has yet been suggested for the poly(A) in mitochondrial mRNA, its presence is clearly unrelated to transport and may be solely intra-mitochondrial, perhaps related to whatever function it has in the cytoplasmic messengers. Also of importance is the observation that the poly(A)-containing mitochondrial mRNA can be fractionated into some eight species, which together with the tRNA and rRNA sequences previously identified may account for transcription of the complete mitochondrial genome. These experiments suggest a view of the mitochondrion as an entity whose evolution has to some extent been independent of the surrounding cytoplasm.

Extending their previous studies to report now the first determination of the location of specific sequences within the non-messenger parts of nuclear HnRNA, Molloy, Jelinek, Salditt and Darnell (*Cell*, **1**, 43; 1974) suggest a tentative structure for the large precursor. That the sequence destined to become mRNA lies at the 3' end of the molecule to which poly(A) is added is reasonably well established. But a crucial question concerns the composition of the remainder of the molecule, which is to be degraded. It is especially important to identify the junction between the mRNA and non-mRNA regions and to establish the criteria used by the maturation system for selecting the messenger.

The technique used by Molloy *et al.* was to isolate poly(A)-containing HnRNA on poly(U) sepharose columns, break these molecules with alkali, and then reisolate the 3' terminal sequences (which still contain poly(A)). By examining the properties of fragments of different lengths, it is possible to deduce the nature of the sequences located on the 5' side of the messenger part of the molecule.

Because HnRNA can hybridise to DNA at rates more rapid than mRNA, it appears to contain transcripts of repetitive sequences absent from the messenger sequences. Using fragments isolated by the alkaline cleavage of HnRNA, Molloy *et al.* found that the first 3,000–4,000 3' nucleotides hybridise at the same rate as mRNA, but that as the fragment length increases to 8,000 nucleotides the rate increases 3–4 times to the value characteristic of HnRNA. Since mRNA is in general about 3,000 nucleotides long, this result implies that the HnRNA molecule

must contain repetitive sequences fairly near to the 5' end of the mRNA section. Because the rate of hybridisation remains constant from a fragment length of 8,000–30,000 bases, the rapidly hybridising regions in this part of the HnRNA may be about equally interspersed with less rapidly hybridising regions.

Another marker used to pin down specific parts of HnRNA is the presence of oligo(U) fragments, generally about thirty bases long. The poly(A) terminated HnRNA molecules larger than some 20,000 nucleotides possess about three oligo(U) fragments each; shorter HnRNAs possess a smaller proportion of these sequences; and mRNA lacks them altogether. More than 90% of the oligo(U) segments are located further than 12,000 nucleotides from the 3' terminus and may be clustered at closely located sites.

A generalised structure for HnRNA thus locates mRNA and poly(A), occupying about 3,000 nucleotides, at the 3' end. Pointing to the observation that rapid hybridisation of HnRNA depends on both cleavage and denaturation, Molloy *et al.* suggest that hairpin structures, in general less than 500 bases in length, may occupy the sequences up to about 15,000 nucleotides from the 3' terminus. Sequences of oligo(U) may then be located towards the 5' end of the HnRNA.

Whereas the addition of poly(A) seems to be a feature distinguishing the mRNA sequences of HnRNA which are to be conserved, a different mechanism has been known for some time to apply to ribosome RNA in eukaryotes. A large precursor, sedimenting at 45S in mammalian cells, is methylated only in certain regions; and these regions ultimately become the 18S and 28S rRNAs. The rest of the molecule is degraded. Methylation has until now been thought to be a prerogative of rRNA and tRNA (in which it is necessary for the functions of the molecule) alone. But Perry and Kelley (*Cell*, **1**, 37; 1974) now provide evidence that mRNA of mouse L cells is also methylated. After labelling with ³H-methionine and ¹⁴C-uridine, the ³H/¹⁴C ratio of the poly(A)-containing RNA of the polysomes is about one fifth of that of rRNA. From this comparison, Perry and Kelley calculate that the proportion of methyl groups in mRNA is about 0.22%. This means that an average mRNA of 3,000 nucleotides should bear six to seven methyl groups.

Both base and ribose moieties suffer methylation, the proportion of base methylation in mRNA being greater than in rRNA. A preliminary analysis suggested that there may be only one

Solar neutrino problem still unsolved

A FEW months ago, Prentice suggested that the absence of neutrinos from the Sun could be explained if that star has a hydrogen-free core amounting to about 0.02 or 0.03 $M_{\odot}$ (*Mon. Not. R. astr. Soc.*, **163**, 331; 1973). Such helium inhomogeneities reduced the neutrino flux by a factor of 10, or least for the simple polytropic models used by Prentice. But now more detailed calculations by Demarque *et al.* (*Mon. Not. R. astr. Soc.*, **165**, 19P; 1973) suggest that this is not the case for realistic solar models.

These detailed models confirm Prentice's qualitative discovery, and show that both central temperature and ⁸B neutrino flux are reduced, with a minimum for a core mass of 0.025 $M_{\odot}$; however, this minimum is only 20% less than the 'normal' flux of ⁸B neutrinos. Even so the results for idealised static models are still better than those obtained when an evolutionary sequence is calculated. A model evolved from a zero age situation in which the helium core was 0.025 $M_{\odot}$ reached one solar luminosity after 4.7×10^9 yr, when the ⁸B neutrino flux was only 1.5% below that for a 'standard' Sun evolved from a homogeneous zero age state.

or a few species of methylated base in the messenger. Some data also suggest that the methylated bases may be concentrated at adjacent nucleotides, perhaps located towards the 5' end of the molecule; and Perry and Kelley suggest that the methylation sites are probably located in non-coding sequences of the messenger.

The proportion of methylated bases in HnRNA is much lower than in mRNA, being barely detectable. It seems possible therefore that methylation may take place in the HnRNA precursor only in those sequences destined to become mRNA, a situation analogous to the maturation of ribosomal RNA. What part this methylation plays in messenger selection must of course at present be a matter for speculation, but it is tempting to suppose that it may be involved in the selection of messenger sequences for conservation and transport to the cytoplasm.

The rationale for the evolution of the complex processing systems for both nuclear mRNA and rRNA remains elusive; it is difficult to see why half of the rRNA precursor and an even greater proportion of the HnRNA precursor should be synthesised only to be degraded. That the maturation process may be part of a system for gene control—rather than simply a feature of the mechanism of mRNA production—remains an intriguing possibility. But the presence of poly(A) in mitochondrial mRNAs throws a puzzling light on its relationship to this process, although methylation now also becomes a candidate for the role of ensuring specificity of selection. Although premature at present, it is tempting also to conclude that a general structure may be valid for all HnRNA molecules so that its features too may be important in regulating gene expression in higher cells.

Controlling toxicity of chromate waste heaps

from our Plant Ecology Correspondent

REVEGETATION of toxic spoil from industrial processes can be achieved only when the true nature of its toxicity has been established. This fact was ably demonstrated by Breeze's preliminary work on the chromate waste heaps of the Croal Valley in Lancashire (*J. appl. Ecol.*, **10**, 513; 1973). Gemmel (*Environ. Poll.*, **5**, 181; 1973) has now found that the interaction of various ions in the chemically diverse waste material of these chromate spoil heaps necessarily complicates any model of toxicity in this system.

Gemmel's technique involved the collection of samples from the waste heaps,

analysing them chemically, diluting them with lime-free sand and testing their toxicity biologically using white mustard (*Sinapis alba*) as a test plant. In chemical analysis he found that levels of chromate, calcium, sulphate, hydroxide and bicarbonate ions were all high enough in the unweathered material to be possible causes of toxicity; in weathered waste magnesium ions also reached high concentrations.

Alleviation of toxicity before revegetation requires the determination of which of these ions is most important in the inhibition of plant growth. Gemmel attempted this by growing mustard on samples of known chemical composition and analysing the data so produced by means of correlation matrices linking dry matter production with the levels of the ions of potential interest. Highest toxicity was correlated with chromate and bicarbonate. Magnesium and calcium ions were positively correlated with dry matter production, indicating that these may alleviate the effects of the toxic ions. This is of particular interest since calcium is known to reduce the toxicity of heavy metal cations, such as lead (Wilkins, *Nature*, **180**, 37; 1957).

If, as seems likely, toxicity is broadly determined by chromate but is modified by other ions at any given chromate level, analysis of data by simple correlation is inadequate. Gemmel tries to overcome this by grouping his samples on the basis of their chromate content together with other criteria and subsequently submitting the groups to multiple discriminant analysis to examine their validity. He then produces correlation matrices for ions and dry matter production of mustard within the groups. These indicate that hydroxide ions exert a synergistically toxic effect at medium and high chromate concentrations.

This approach, however, does not overcome the disadvantages of relying on correlations in what is evidently a chemically complex situation; for example, positive correlations between certain ions, such as magnesium and bicarbonate in weathered waste, may result from a common origin in the spoil, in this case magnesium hydroxide, reacting with carbon dioxide. If one of these ions should exert an influence on and therefore be correlated with plant growth, the other is bound to show a similar effect in terms of correlation.

As in all such multivariate situations, the ultimate solution lies in an experimental design which will allow the manipulation of the concentrations of individual ions while other ions deemed critical can be held constant. Without such an approach knowledge of the key factors controlling toxicity in waste materials must continue to be speculative.

Rhythms and the Earth's rotation

from a Correspondent

TIME is simply a progression of events, so that different clocks measure different things and their comparison gives a vital synthesis to all the sciences, even if the philosophical puzzle of the real nature of time still remains. A conference organised by G. D. Rosenberg and S. K. Runcorn on January 8–10, in the School of Physics of the University of Newcastle upon Tyne, on biological clocks and the rotation of the Earth, considered comparisons between atomic, astronomical and biological clocks and the ways in which these give information, not only on the changes in the length of the year and lunar month, but on basic organic processes, the evolution of the Solar System, changes in physical 'constants', and so on.

Several speakers (G. R. Clark, University of New Mexico; J. W. Evans, Memorial University of Newfoundland; C. Hall, University of California, Los Angeles; Rosenberg; and M. A. Whyte, University of Hull) were concerned with recent experiments to determine when organisms, particularly marine bivalves and corals (R. W. Buddemeier, University of Hawaii) actually put on growth increments. It seems strange that such vital aspects have been so little studied as it is of obvious importance that some organisms show daily increments and others show additional tidal, seasonal, reproductive and circadian growth rhythms. Different animals were discussed in which one or more of these patterns could be determined, mostly by optical means, although it was sometimes necessary to resort to staining or X-ray analyses. But I. Thompson (Princeton University) showed that even when isolated from all obvious environmental stimuli, including food, some bivalves seem to regulate their biological clock with the movements of the Moon, presumably through changes in the gravity field or electromagnetic changes, so that marine organisms well below the tidal and even the photic zones may still show externally related growth rhythms.

Such fundamental findings have already become important to palaeontologists, because observations of past growth bands can determine past environments, not only on a gross scale, by the palaeolatitudinal implications of either seasonal banding or the thickness of the incrementals, but also in terms of past water depths, compositions of the substrate and local ecological relationships. The greatest interest, however, was in new results obtained by W. B. N. Berry (University of California) and G. A. L. Johnson (University of Dur-

ham) from counts of different types of increment shown by a variety of fossils several 100 million years old which have enabled the number of days in the month and in the year to be determined. Calculations from stromatolites more than 2,000 million years old have also been made by G. Pannella (University of Puerto Rico) and R. E. Mohr (University of Minnesota). It turned out that many counts have, in fact, been made, but only a daring few had, as yet, been published, mainly from an uncertainty of either their meaning or reliability. Yet there was remarkable agreement that these counts showed that Earth had spun faster in the past and that its run-down has not been constant, but decelerated very slowly through most of the Precambrian and much of the Phanerozoic. Alterations in the rate of deceleration possibly correlate with geological or geophysical changes.

The problems of counting how many lines actually exist, or seem to exist, between yet another series of bands of varying distinctness, was in stark contrast to the precision of astronomical timing. Since 1955 the atomic clock has enabled incredibly accurate measurements of the rate of the Earth's rotation to be made and has already confirmed the somewhat less precise measurements described by N. P. J. O'Hara (Royal Greenwich Observatory) on the observations of the changing positions of the planets against the stars. L. V. Morrison (Royal Greenwich Observatory) demonstrated how these results show a secular deceleration of the Earth's rotation during the past 250 years of 42 ± 6 seconds of arc century⁻² (2 ms century⁻² of time). Superimposed on this are irregular fluctuations which were attributed to wind patterns in the short term and to changing electromagnetic coupling between the Earth's core and mantle for longer periods—although doubts were expressed as to whether this force was really adequate.

F. R. Stephenson and P. M. Muller (University of Newcastle upon Tyne) presented results culled from mediaeval chronicles, the classics, Babylonian and ancient Chinese manuscripts and showed that total eclipses during the past 2,500 years indicated a uniform secular deceleration of about 83 arc s century⁻² for the Earth and 32 arc s century⁻² for the longitudinal position of the Moon. Such rates extended over geological time, however, would result in the collision of the Earth and Moon only 600 million years ago, which is inconsistent with the geological evidence. K. M. Creer (University of Edinburgh), J. A. Jacob (University of Alberta) and Runcorn, with J. Gribbin (*Nature*), J. Keeney (Superior Oil), D. H. Tarling (University of Newcastle upon Tyne), D. H.

Weinstein (Superior Oil) and P. Wesson (University of Cambridge) all offered possible explanations of varying credibility for the variation in the slow-down of the Earth, which was attributed to a lack of deep oceans in the past, changes in the atmosphere or geomagnetic field, to changes in either the gravitational or Hubble constants (G and H). The final conclusion was that biologists should try to find which organisms best represent which rhythm and why, so that palaeontologists can then determine which fossils to study to enable geologists and geophysicists to eliminate at least some of their variables.

Membrane components in virus infected cells

from a Correspondent

How do cell membranes assemble? This question is obviously unanswerable at present, but the meeting of the Cell Surface Discussion group that took place on December 11 at the University of Warwick was centred on a model system that may well provide important evidence leading to a correct solution of this problem. The meeting, organised by Professor D. C. Burke, was concerned with biosynthesis of membrane components in virus infected cells.

Participants at the meeting, by no means all virologists, were introduced by R. W. Compans (Rockefeller University, New York) to the many attractions of the viral system. The final step in enveloped virus maturation (for example, simian virus SV5) involves budding of nucleocapsids through modified regions of the surface membrane of the permissive host cell, modified, that is, by the insertion into the membrane of several viral gene products. These products include polypeptides which are glycosylated by host cell glycosyl transferases and form the glycoprotein species of the envelope of fully assembled virions. During virus replication, the synthesis of host cell proteins is often severely limited or shut off altogether. Development of surface membrane 'patches' of relatively simple composition—in most cases one or two virally coded glycoproteins—can therefore be studied in infected cells. The patches can be identified by specific antibodies directed against the virus. Using antibodies tagged with ferritin, very clear areas of the host cell membrane are visualised in this way.

Insertion of the virally specified antigens into the plasma membrane of the host cell may occur in a single step by fusion with the plasma membrane of an intracellular vesicle containing viral glycoproteins. Alternatively, in-

sertion of the first viral antigen into the host cell membrane forms a nucleation point for the insertion of other neighbouring viral antigens. Yet another possibility is that insertion is random all over the host cell surface and redistribution takes place in a later step in viral maturation. The latter hypothesis invokes the by now familiar principle of membrane fluidity and migration of large molecules in the plane of membrane structures.

Membrane fluidity of lectin-binding sites in cells infected with budding viruses was discussed by P. Reeve (University College Hospital Medical School, London) and H. Becht (Justus-Liebig University, Giessen). Concanavalin A and wheat germ agglutinin (WGA) agglutinate cells infected with certain orthomyxoviruses and paramyxoviruses, and block virus release from these cells. Since infected cells bind no more radio-labelled concanavalin A molecules than do uninfected cells that do not agglutinate, agglutination is believed as in other systems to result somehow from clustering of surface membrane glycoproteins and is not due to the exposure of new lectin-binding sites in infected cells, at the budding sites for instance. Concanavalin A also agglutinates viruses such as fowl plague but not protease-treated virions from which the surface carbohydrate has been removed. In the latter case, however, receptor sites are now exposed for binding to *Dolichos biflorus* lectin. Becht suggested that these sites are present in glycolipids containing N-acetyl galactosamine.

If membrane fluidity and redistribution of viral glycoproteins turns out to be the correct mechanism by which viral specific patches form in the surface membrane of infected host cells, the way in which the redistribution is directed in viral maturation remains unclear. Association of viral proteins molecules (matrix M protein) on the cytoplasmic side of membrane patches containing viral antigens was described for SV5 by R. Cartwright (Animal Virus Research Institute, Pirbright), for VSV and for fowl plague virus by J. Skehel (National Institute for Medical Research, Mill Hill). These molecules were not seen associating with other areas of the host cell membrane containing no viral envelope antigens. It is possible that it is this association which induces formation of the 'patches' rather like the interaction of spectrin and the major erythrocyte glycoprotein. Compans, however, reviewed the evidence that in many viruses the envelope glycoproteins do not penetrate the lipid bilayers to any great extent and certainly do not span it. If this is true also for the 'patches', the question that then arises is what is the recognition

mechanism by which viral core proteins station themselves immediately on the cytoplasmic side of areas of the host cell membrane containing externally-oriented viral glycoproteins? The specificity presumably must lie in the lipid composition of these membrane patches.

Skehel reported that during infection of chick cells with fowl plague there is a build up of viral glycoproteins in the host cell plasma membrane. Insertion of large amounts of these molecules, the viral haemagglutinin and neuraminidase, into the membrane is not compensated for by a loss of host cell membrane components: all of the host cell polypeptides can be identified in apparently normal amounts in the plasma membrane fraction of virally infected cells. Again, however, as in the SV5 system the virally coded glycoproteins form discrete clusters. This was shown after sonication of plasma membrane preparations of infected cells to form vesicles. A vesicular subfraction absorbed preferentially to red blood cells and contained in addition to the expected viral haemagglutinin, the matrix protein and neuraminidase. Moreover, some host cell membrane proteins were present. By contrast, an unabsorbed subfraction contained in addition to host cell membrane proteins, the viral neuraminidase and the uncleaved haemagglutinin.

The mechanism of the cleavage process of viral envelope precursor polypeptides received considerable discussion. Perhaps the most thoroughly studied system is in cells infected with Semliki Forest virus. S. I. T. Kennedy (University of Warwick) summarised the work of the Warwick group and of other workers on the replication of Semliki Forest virus, an alphavirus, in BHK cells. When infected cells are treated with protease inhibitors (TPCK), several viral gene products accumulate intracellularly. The largest of these is a polypeptide of molecular weight 160,000, a value that is a little short of the translational product expected of the major (26S) RNA present in virally infected cells. The polypeptide normally undergoes a series of proteolytic cleavages to produce first a polypeptide of molecular weight 120,000 and finally a core protein (molecular weight 35,000) and two envelope glycoproteins EP1 and EP2 (molecular weights 57,000 and 54,000). Intermediate partially cleaved polypeptides have also been identified. Pactamycin 'blocking' experiments indicate that the core polypeptide is N-terminal to the envelope polypeptides EP1. EP2. A small carbohydrate rich fragment is apparently inserted between EP1 and EP2 in the uncleaved precursor and is cut out during the fashioning by limited proteolysis.

R. Weiss (Imperial Cancer Research

Fund, London) argued for the presence of RNA tumour virus antigens on some apparently normal chick embryo cells. Cells (GS⁺) genetically endowed with the ability to synthesise this surface antigen, a glycoprotein support the production of infective virus (Bryan strain of RSV) whereas negative (GS⁻) chick cells do not. The antigen is incorporated into released virus and is necessary for subsequent attachment of virus to new host cells. Two other products studied by Weiss are coded by host genes; one of these is a surface receptor recognised by the GS⁺ product. The other host gene specifies a surface protein product that prevents infection by blocking the receptor site when this is present. Attempts to expose the receptor in blocked cells by mild proteolysis have been unsuccessful. The inhibitor gene segregates with the GS⁺ gene and it is possible that the two functions represent pleiotropic expressions of the same gene.

Cytochemical approach to hormone assay

from a Correspondent

THE main conclusion of the symposium on the cytochemical approach to hormone bioassay, held at the Kennedy Institute of Rheumatology in London on December 6, is that quantitative cytochemistry is now so precise that it can be used to measure the response produced by as little as 50 fg ml⁻¹ of a hormone acting on its target cells. Consequently even 0.1 ml of plasma is sufficient for measuring the content of corticotrophin in the blood of the patient or animal; the plasma sample is measured at two dilutions (1:100 and 1:1000) and the results should agree to $\pm 15\%$.

Cytochemical methods for measuring the effect of four hormones are now in use. The first method, for the bioassay of corticotrophin (ACTH), is the most fully developed and the whole morning was devoted to the technique and its applications. The first part of the afternoon was concerned with the development of this method to the bioassay of luteinising hormone (LH) and with preliminary results concerning the levels of this hormone during the menstrual cycle. The second part of the afternoon was devoted to two of the more recent investigations—studies on the effect of thyroid stimulating hormone (TSH) and the long-acting thyroid stimulator (LATS)—and those which may lead to a new assay for gastrin.

Essentially the methods for all these assay studies are similar. The target tissue is removed from the animal and

cut into segments. Each segment of tissue is maintained for 5 h in non-proliferative maintenance culture. This manoeuvre removes the target cells from the hormone influence of the animal and allows them to recover from the trauma of excision. The culture medium is then removed and replaced for a matter of only minutes with culture medium containing either graded concentrations of the standard hormone preparation (to obtain a response: concentration calibration graph) or one of two dilutions of the unknown plasma. The tissue is chilled, sectioned and reacted by a cytochemical method for the most appropriate biochemical activity affected by the hormone. Much of the sensitivity of these assays depends on the use of scanning and integrating microdensitometry for measuring this response solely in the target cells; moreover, they are within-animal assays so eliminating the notorious biological variability of most bioassays. The speed with which at least one of these assays can be performed has been increased remarkably by a new modification in which 20 μ m sections are used instead of segments of the target tissue.

The past ten years have seen a revolution in endocrinology caused by the advent of radioimmunoassay of hormones. This technique, however, has two drawbacks. In the case of certain hormones, it is very difficult to measure low physiological, or subnormal, concentrations by such methods and they sometimes detect immunopotent molecules which may have little or no biological activity. The advantages of the cytochemical bioassays are that they easily encompass the measurement of such subnormal levels and, of course, measure only biologically active molecules. In the case of ACTH the divergence of the radioimmunoassay and bioassay results became marked only during the rapid rise and fall which occurred during the hypoglycaemia stress tests, done to determine how well the hypothalamo-pituitary-adrenal function was maintained in rheumatoid patients treated with ACTH or with corticosteroids. A more striking divergence was seen in the levels of LH; this, if fully established, could have important implications in the present understanding of ovulation.

The techniques were first developed in the Division of Cellular Biology at the Kennedy Institute in collaboration with the Department of Chemical Pathology of the Charing Cross Hospital Medical School. They were then extended by the Department of Chemical Pathology at St Bartholomews Hospital Medical College and are currently also in use in the Department of Pharmacology, the Royal Free Hospital Medical School, and in the Department of Medicine at Newcastle.

A case for regional culture collections of microorganisms

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Dr Martin describes the proposals for regional culture collections of microorganisms especially in developing countries.

UNTIL fairly recently, little attention has been paid to the diminution of the genetic pools of higher plants and animals. On the other hand, since the earliest days of their science, microbiologists have been forced to conserve the genetic resources of their microbes, albeit they have not always expressed their concern in terms of preservation of genetic pools. Microbiologists, of necessity, have had to preserve their cultures in a viable and physiologically unaltered state since relatively little can be learned from dead microorganisms. As a consequence, a number of very fine culture collections have been established and considerable research effort has been expended on developing techniques suitable for the long-term storage of living microbes.

In 1962 a meeting of culture collection specialists (266 scientists from twenty-six countries) was held in Ottawa, Canada. Recognising the vital role of culture collections in the science and technology of microbiology, the delegates recommended that International Association of Microbiological Societies (IAMS) establish a section on culture collections. IAMS acceded to this request at its next meeting and the first formal meeting of the Section (later to become the World Federation for Culture Collections [WFCC]) took place in Paris in 1966. In 1968 an international conference on culture collections took place in Tokyo. In attendance were 300 scientists from fifty-two countries, twenty-seven of which could be classed as developing. A third conference was held in July 1973 in Sao Paulo, Brazil.

Parallel to these developments, the Japanese Federation of Culture Collections in 1963 submitted, through the Japanese delegation to Unesco, a proposal for a 10-yr programme for research in microbiology. Heavy emphasis was placed on the need for an international federation of culture collections and for the establishment of specialised culture collections throughout the world. The proposal was referred to IAMS and in 1964 IAMS presented its brief to Unesco. Unesco initiated action by convening a meeting of culture collection specialists in Paris in 1966.

Brief to UN

Over the past decade, WFCC has given a great deal of consideration to problems faced by culture collections and has initiated a number of relevant programmes. Thus, it was that WFCC found itself in an excellent position to submit to the secretariat of the UN Conference of the Human Environment (Stockholm 1972) a brief on the conservation of genetic pools of microorganisms. The main points made in the brief were: (1) In any consideration of the conservation of the genetic resources of the world, it is imperative that the need to conserve the genetic pools of microorganisms be recognised. (2) Man, and other forms of life, could not exist without microorganisms since it is through their diverse activities that the essential elements for nature are recycled. Without this recycling, all life forms would soon die. (3) Al-

though the greatest amount of microbial activity goes on in nature without the guiding hand of man, man does apply microorganisms in a great many ways for his own betterment. (4) Wherever and whenever microorganisms are applied scientifically for man's benefit, it is essential to have available a large number of diverse sources of microorganisms. (5) Culture collections are 'libraries' or 'museums' of living microorganisms, where cultures are received, maintained in a viable and genetically unaltered state and studied, and from which cultures are dispatched to other workers. (6) Culture collections also act as clearing houses for information concerning the cultures they maintain. (7) Culture collections quite often provide identification of newly isolated strains of microorganisms.

As a result of the WFCC and other similar briefs, a series of recommendations dealing with the conservation of the genetic resources of microorganisms came out of the Conference.

To some it was difficult to see how the UN would organise the proposed coordination of collections of microorganisms. To others, it was difficult to understand why the conservation of microbial gene pools should be of concern when there already exist a number of fine collections of cultures of microorganisms. "It is unrealistic to establish culture collections in developing countries" is a statement one hears voiced rather frequently. Such a statement can be justified only if by it one means that it is unrealistic to maintain a large service collection, such as the American Type Culture Collection (ATCC) or the National Collection of Type Cultures (NCTC), in every developing country in the world. To accept the statement at its face value would be to take the position that it is unrealistic to carry on work with microorganisms in developing countries.

What then is a culture collection? It is precisely what the term implies: a collection of cultures of living microorganisms, with no constraints as to number of strains or types of microorganisms held. Such a broad definition is necessitated by the fact that the same principles apply to the conservation of a single strain as to the conservation of many. Furthermore, the preservation of microorganisms in a healthy and physiologically unaltered state is, or should be, of equal concern to the worker who uses only a few strains as it is to the curator of a large service collection. With this basic definition in mind, we can then logically recognise several types of culture collection ranging from the large service collection, such as ATCC or NCTC, whose prime function is the supply of cultures on demand, to the small private collection which may or may not distribute its cultures. Between these extremes lie a host of intermediates.

Plan of action

In August 1972, WFCC submitted to the conference secretariat an 'Action Plan' for the implementation of the recommendations made at Stockholm. The following is an interlock of the pertinent recommendations and the WFCC proposals:

"It is recommended that governments agree to an international programme to preserve the world's genetic resources.

- Active participation at the national and international levels is involved.
- An international network is required with appropriate machinery to facilitate the interchange of information and genetic material among countries.
- Both static (seed banks, culture collections, etc.) and dynamic (conservation of populations in evolving natural environments) ways are needed.
- Action is necessary in several inter-related areas:
 - (1) survey of genetic resources
 - (2) inventory of collections
 - (3) exploration and collecting
 - (4) conservation
- Although the international programme relates to all types of genetic resources, the action required for each resource will vary according to existing needs and activities."

WFCC is fully prepared to serve as the microbiological partner in the international programme to preserve the world genetic resources.

"Compile or extend, as necessary, registers of existing collections.

- In respect to microorganisms, it is recommended that each nation develop comprehensive inventories of culture collections:
 - (1) a cataloguing of the large and small collections and the value of their holdings is required, rather than a listing of individual strains
 - (2) many very small but unique collections, sometimes the works of a single specialist, are lost
 - (3) governments should assure that valuable gene pools held by individuals or small institutes are also held in national or regional collection."

WFCC has already prepared an extensive documentation on large and small collections in all parts of the world and their holdings. WFCC proposes to expand and continually update this information and to make it available to governments and international, regional and national organisations.

"Microorganism germ plasm: cooperatively establish and properly fund a few large regional collections.

- Full use should be made of major collections now in existence.
- To provide geographic distribution and access to the developing nations, regional centres should be established in Africa, Asia and Latin America and the existing centres in the developed world should be strengthened."

In conjunction with IAMS and the International Cell Research Organisation/Unesco Panel for Microbiology and in consultation with other UN agencies, WFCC proposes setting up a working party to be charged with the following: (1) Choosing suitable sites for regional culture collections in Africa, Asia and Latin America. (2) Providing guidelines for the establishment of these regional collections. (3) Establishing mechanisms for ensuring that continued quality and functioning of these collections is maintained.

"Collaborate to establish a global network of national and regional institutes based on agreements on the availability of material and information, on methods, on technical standards, and on the need for technical and financial assistance wherever required.

- Such cooperation would apply to all types mentioned above.
- Standardised storage and retrieval facilities for the exchange of information and genetic material should be developed:
 - (1) information should be made generally available and its exchange facilitated through agreement on methods and technical standards
 - (2) international standards and regulations for the shipment of materials should be agreed upon

- (3) basic collections and data banks should be replicated in at least two distinct sites, and should remain a national responsibility
- (4) standardised and computerised system of documentation is required.

- Technical and financial assistance should be provided where required:

- (1) areas of genetic diversity are most frequently located in those countries most poorly equipped to institute the necessary programmes."

Based on its extensive experience in the field of the distribution of cultures and of information pertaining thereto, WFCC considers itself competent to contribute to the standardisation of storage and retrieval facilities for the exchange of information and microbial genetic material. The necessary framework is already set up. WFCC could contribute, through its committee on documentation, to the establishment of "a standardized and computerized system of documentation". The information already collected, and to be collected in the future, is readily available to all.

WFCC has set up a working group to study "international standards and regulations for the shipment of materials".

Regional centres

The maintenance of data and important cultures in more than one centre is one of the guiding principles of WFCC.

"The need for liaison among the parties participating in the global system of genetic resources conservation requires certain institutional innovations.

- It is recommended that the appropriate United Nations agency initiate the required programme on microorganism germ plasma
- periodic international conferences involving those concerned with the maintenance and research on gene pools of microorganisms should be supported
 - (1) such a programme might interact with the proposed regional culture centres by
 - (a) assuring that each centre place high priority on the training of scientists and technicians from the developing nations
 - (b) acting as a necessary liaison
 - (c) lending financial assistance to those collections established outside the developed countries
 - (2) the international exchange of pure cultures of microorganisms between the major collections of the world has operated for many years and requires little re-enforcement
 - (3) study should be particularly conducted on waste disposal and recycling, controlling diseases and pests, and food technology and nutrition.

This recommendation relates directly to the existing programme of conferences and training courses of WFCC. The WFCC is prepared to undertake expansion of this programme to ensure interaction with the proposed regional culture collection centres.

It is worth noting at this point that the concern for culture collections in developing countries has not been exclusive to the WFCC and curators of culture collections. In the *Report and Proceedings of the Expert Group Meeting on the Manufacture of Chemicals by Fermentation*, Vienna, 1969 (United Nations Industrial Development Organisation publication E.71.II.B.6) it was recommended that

"A few regional culture collections should be established, which would supply cultures and transmit information and technical expertise relating to cultures. These should be located in the vicinity of fermentation plants or universities doing applied fermentation research. Staff members should be available capable of solving microbiological problems in the plants of their regions. These centres might even supply inoculum to plants of their regions. They would also be good places to train tech-

nicians in production control and techniques (determination of pH, product stability, etc.). Training would last up to one year. Probably centres would be needed currently in Central Africa, India, the Middle East, North Africa and South America."

Unfortunately it seems that UNIDO failed to act on this recommendation.

Developing countries

It is most reasonable to suggest that major sources of microorganisms, the large service collections, should be limited to relatively few well equipped and well supported institutions strategically placed throughout the world. (At this point it is perhaps well to re-emphasise that culture collections do much more than simply distribute cultures; they also act as important identification, information and training centres.) Unfortunately, the large service collections are located almost exclusively in the highly industrialised nations. For reasons of either cost or distance, workers in developing countries are frequently denied these goods and services. Furthermore, it must be recognised that, although service collections operate on an international basis, they are in fact set up to meet primarily national needs. Indeed the very name of the collection usually carries some designation to the effect that it is a national collection. Developing countries have their own peculiar problems and there is need for service collections which specialise in those organisms and services having relevance to these areas.

WFCC feels that the proposed regional culture collections should concentrate on the maintenance of strains of microorganisms which are related to problems of the region. Furthermore, regional collections, wherever possible, should be located in existing research centres concerned with work

relevant to the cultures maintained and they themselves should be focal points for research and training. One can envisage a regional collection as being composed of several specialised sub-collections each located in a different institution but functioning as a unit.

Personnel of the proposed regional centres must be well trained in all aspects of the organisation and operation of culture collections. Senior scientific personnel should have the opportunity to spend approximately a year working in one or two major collections in the developed world before the centres actually become operational. Technical personnel should attend at least a 2-3 week group training course in culture preservation. Once the regional centres are fully operational, they should act as centres for training microbiologists of the region. As regards the importance of the proposed regional culture collections as training centres, it should be emphasized that the conservation of microorganisms is properly as much a matter of concern for the microbial biochemist or the fermentation technologist who uses only one or two organisms as it is for the curator of a large service collection.

It is obvious that any consideration of culture collections should be made in the overall concept of microbiology. The collection staff should consider itself part of a regional 'microbiology team' concerned with the application of the activities of microorganisms to the fulfillment of human social and economic needs. Thus the centres can make a significant contribution to upgrading all aspects of microbiology in developing countries.

In summary, it does seem reasonable that the UN initiate the formation of regional culture collections in the third world, since the need exists and the process could be carried forward in a rapid and logical fashion.

Tectonic evolution of continents in early Proterozoic times

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In early Proterozoic times, complex systems of mobile belts were developed within large continental masses as a result of the internal distortion of these masses which accompanied bodily movement relative to the poles. The arrangement of structures formed by these processes differs from that characteristic of both Archaean and Phanerozoic tectonic regimes.

THE portions of the continental crust which acquired their tectonic patterns in early Proterozoic times show a structural style which differentiates them on the one hand from Archaean provinces whose structures record widespread mobility and on the other from late Proterozoic and Phanerozoic provinces whose structures record the disruption and collision of rigid plates. Palaeomagnetic evidence^{1,2} suggests that over the period from about 2,500 to 1,300 Myr ago crustal masses of continental dimensions were moving as units relative to the poles³. The arrangement of certain major structural features also points to the existence at this period of large sheets of continental crust^{4,5}. There

is, however, abundant evidence that these sheets did not behave like the rigid lithosphere plates of the present day: a glance at the relevant tectonic maps shows that they were mosaics in which more stable massifs were enclosed by more mobile belts. Although early Proterozoic plates moved as coherent entities, they were not rigid and suffered extensive internal deformation and metamorphism while in transit. We propose to examine here the structural patterns which resulted from this distinctive style of tectonics.

North America

The massifs which remained relatively stable over the period 2,500-1,700 Myr—the Superior, Slave and eastern Nain provinces—are set in a network of mobile belts which includes the Churchill province and its extensions (Fig. 1). Marginal structures comparable with those developed at younger destructive plate boundaries mark the Labrador and Coronation geosynclines where Proterozoic fold belts incorporating eugeosynclinal assemblages flank the stable massifs^{7,8}. Elsewhere, however, the marginal structures of the massifs are of a different type. The majority are defined by tectonic lineaments belonging to a widely developed

north-easterly set, locally marked by lines of intrusions, by mineralised zones or by narrow tracts of deformed sedimentary rocks. The distortion of earlier structures in these lineaments suggests that their formation was associated with transcurrent displacements of up to a few hundred km².

Plotting of the north-easterly lineaments on a globe shows that they fall on small circles about an axis lying near the west end of the Aleutian arc. Regional dyke-swarms dated at (2,500) 1,800 Myr in the Slave province and the Ungava peninsula of the Superior province^{8,9} lie close to a great circle passing through a point only some 20° from this axis and may record extensional rotation. If we regard these displacements as adjustments to the bodily motion of the continental sheet, it seems reasonable to infer that the sheet was being rotated about the same axis. A clockwise movement parallel to the small circles defined by the transcurrent lineaments seems compatible with the appropriate polar wander path (track 5, 2,500–1,950 Myr) of Donaldson *et al.*² Figure 1 shows that movements parallel to the lineaments would be capable of opening or closing oceanic basins on the sites of the Labrador and Coronation geosynclines which are set at an angle to them. We therefore envisage a situation in which the precursor of North America moved westward, perhaps in response to the opening of a major ocean on its north-eastern flank and was simultaneously distorted by displacements on deep transcurrent faults and by the jostling of the stable provinces against their more mobile surroundings. The short eugeosynclinal welts may mark 'tears' linked by north-easterly transform faults, along which small oceans were formed and subsequently destroyed. A certain amount of further internal deformation was taken up in those portions of the Churchill province which are occupied neither by geosynclinal assemblages nor by lineament tracts and which were sites of metamorphism and granite formation during the Hudsonian event at about 1,800 Myr. The survival of incompletely modified older tectonic patterns in some of these regions, as indicated by the aeromagnetic map¹⁰, suggests however that deformation was relatively weak.

Distortion and metamorphism continued in the interior of the North American continental sheet until the ending of the Hudsonian event, perhaps changing in style or direction at the time at which the hairpin bend of the polar wander curve² suggests a change in motion of the sheet

as a whole. Structures of new kinds developed from about 1,300 Myr onward include the rift-like Keweenaw and related volcanic troughs, the Muskox basic complex, the Mackenzie dyke-swarm and the Belt sedimentary basin. Taken together with the Gardar igneous province of southern Greenland, these features—mostly suggesting extensional movements—define an incomplete polygonal pattern avoiding the oldest stable massifs. The nearest analogies seem to be with Phanerozoic rift-valley systems; the polygonal pattern suggests fracturing over domal upwarps around the Great Lakes and Coronation Gulf. Mobile provinces were less extensive than in the preceding period and there is some evidence² suggesting that the principal (Grenville) mobile belt was peripheral rather than internal in position.

Greenland and north-west Britain

The Pre-Ketilidian stable massif of Greenland is enclosed by mobile regions including the Ketilidian and Nagssugtoqidian of Greenland and the Laxfordian of Britain. Bridgwater *et al.*¹¹ and Windley¹² infer that the massif moved relative to its mobile borders and recognise contrasts in its marginal relationships similar in implications though not in detail to those discussed above. On a construction which eliminates the North Atlantic and Davis Strait (Fig. 1) the great shear zones at the north-west side of the Pre-Ketilidian massif fall into place as members of the set of north-easterly transcurrent lineaments developed in North America. The north-westerly shear zones of the Laxfordian complex clearly do not belong to this set which may be represented in Scotland by a steep north-easterly zone of strong deformation which skirts the Outer Hebrides. No eugeosynclinal welts oblique to the principal lineaments are represented. Contrasts between the Ketilidian mobile belt of southern Greenland and the Laxfordian and Nagssugtoqidian provinces suggest that the former, which has been compared with the Andean belt¹³, may be part of a peripheral system and the latter parts of an internal ensialic network.

Gondwanaland

So much of the early Proterozoic provinces in Gondwanaland has been reworked during Mozambiquian or Indian Ocean tectonic events (at about 800–500 Myr) that their early history is obscure. Some structures shown in Fig. 2 are usually regarded as not older than late Proterozoic and may ultimately have to be discarded for the purposes of this discussion. We provisionally recognise in Africa a set of major Proterozoic lineaments including the Ubendian belt, long regarded by McConnell as a product of transcurrent movement¹³, the Aswa zone of Uganda and perhaps the great dislocations of the Hoggar. These lineaments fall on small circles about an axis located near the north-east of peninsular

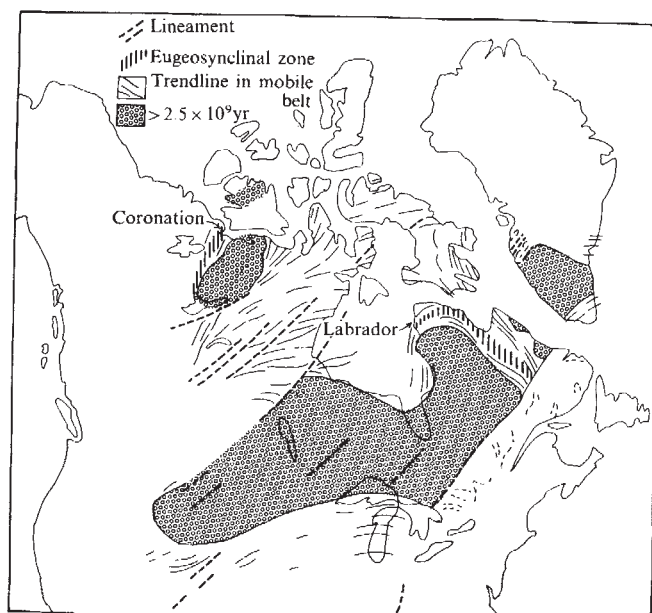


FIG. 1 Some Proterozoic structures in North America and Greenland.

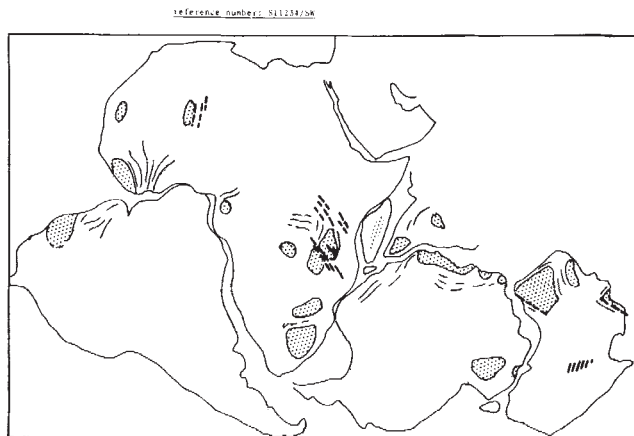


FIG. 2 Some Proterozoic structures in Gondwanaland; symbols as in Fig. 1.

India. Clockwise motion of the continent on this axis would be roughly consistent with the polar wander track for the period 2,340-2,200 Myr¹.

In Australia, narrow north-easterly structures comparable with the transcurrent lineaments of other continents make the boundaries of the Yilgarn and Carpentaria stable blocks along the Frazer Range and the Halls Creek mobile belt. On Smith, Briden and Drewry's reconstruction of Gondwanaland¹⁴ they fit reasonably well on the small circles defined by the African lineaments, as does the main early Proterozoic metamorphic belt of Antarctica¹⁵. These coincidences are consistent with the inference drawn from palaeomagnetic studies^{3,16} that the core of Gondwanaland moved as a unit through much of Precambrian time. Mobile belts such as that of Mount Isa which have geosynclinal characters are, as in North America, oblique to the lineament set and may mark the site of oceans opened by small relative movements of adjacent blocks.

The structural relationships discussed above seem to record an integrated series of relative movements taking place within one or a few large continental sheets which were themselves moving as essentially coherent units. Peripheral mobile belts formed in response to bodily movements of the sheets may be distinguished from interior, ensialic, mobile belts formed in response to comparatively small relative movements of the more stable massifs within them. Deformation in the interior mobile belts together with displacements on major sets of transcurrent lineaments which traversed the continents and acted in some instances as transform faults led to distortion of each continental sheet as a whole and to the opening and closing of small oceanic basins within it. Metamorphism and granite formation widely associated with tectonic activity show that geothermal gradients in the interior network of mobile belts were steep.

The distinctive features of the early Proterozoic tectonic systems are those which record the internal deformation of the large continental plates. Taken in conjunction with the evidence of bodily movements supplied by palaeomagnetic data they allow, as we suggest above, interpretation of Proterozoic tectonics on a global scale.

Received November 22, 1973.

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Methionine transfer RNAs associated with avian oncornavirus 70S RNA

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tRNA free in RNA tumour viruses contains very little tRNA_f^{Met}, whereas tRNA associated with viral 70S RNA contains both tRNA_m^{Met} and tRNA_f^{Met}. The close association of the initiator tRNA with 70S nucleic acid suggests that it may be involved as a primer for reverse transcriptase.

RNA tumour viruses contain two physically separate classes of tRNA. The major class is free within the virion, and contains a selected population of host cell molecules¹⁻⁶. The minor class of 4S RNA is intimately associated with viral 70S RNA, and comprises 2.5-3.0% of the total mass of the aggregate⁷. This 70S-associated tRNA has a minor base composition similar to that of free viral 4S RNA⁸, and contains several amino acid acceptor activities including acceptance of lysine, phenylalanine, proline, histidine, threonine and serine⁹.

Eukaryotic cells contain two types of tRNA which accept methionine. One species is the initiator of protein synthesis and although it is not formylated *in vivo* (as in the initiator tRNA in prokaryotes) it can be formylated by *Escherichia coli* transformylase. It is therefore designated tRNA_i^{Met}. The other tRNA^{Met} species donates its amino acid into internal positions of proteins and is designated tRNA_m^{Met10}.

We have previously analysed the methionine-acceptor activity of the free tRNA of avian myeloblastosis virus (AMV), and shown that 85% of this fraction consists of a single 'm' type species, similar to a corresponding chick cell molecule with respect to chromatography on RPC-5 absorbent, the electrophoretic mobility of the terminal methionyl-oligonucleotide, and its activity in donating methionine into polypeptides synthesised in an ascites cell-free system¹¹. Free viral tRNA contains very little initiator RNA.

We now report a similar study of 70S-associated tRNA^{Met}, and show that this fraction contains both types of methionine-

accepting tRNA. Reversed-phase chromatography on RPC-5 of 70S-associated tRNA charged with ^{35}S -methionine reveals a profile qualitatively similar to that obtained with chick cell tRNA^{Met}, containing one peak of 'f' type activity, peak I, and three peaks of 'm' type tRNA^{Met}, peaks II, III and IV¹¹. Peak I isolated from AMV 70S-associated tRNA is shown to be an initiator; it can be formylated by *E. coli* transformylase the terminal methionyl-oligonucleotide co-electrophoreses with that from Met-tRNA_f of chick and ascites cells, and it responds to the synthetic polymer AUG (U)₃₃ in an ascites cell-free system. Furthermore, 70S-associated Met-tRNA_f donates its methionyl residue into the N-terminal position of nascent peptides synthesised *in vitro* in response to both natural and synthetic mRNAs. The 70S-associated Met-tRNA from peak IV, on the other hand, is identical to the free virion peak IV material and has been characterised as a tRNA^{Met}_m species.

Structure of 70S-associated Met-tRNAs

AMV was purified from the serum of leukaemic 10 to 15-d-old chicks, and Rous sarcoma virus, Prague strain (RSV (Pra)) and Schmidt-Rupin strain (RSV (SR)) were prepared from the supernatant medium of infected secondary chick embryo cells. 70S RNA was purified by published procedures^{1,7}. 4S RNA was released from the 70S RNA by heating to 70°C for 3 min in 10 mM EDTA, 100 mM Tris HCl, pH 7.2. The solution was chilled, and the 4S RNA separated by sucrose gradient centrifugation. Aminoacyl-tRNA synthetases were prepared from chick embryos by the method of Taylor *et al.*² and freed of endogenous tRNA by passage through Sephadex G100. 70S associated RNA was acylated *in vitro* with ^{35}S -methionine (Radiochemical Centre, specific activity 100 Ci mmol⁻¹) and extracted as previously published. Deacylation of the viral tRNA prior to reaction with amino acyl-tRNA synthetase was found to be unnecessary. The separation and isolation of ^{35}S -Met-tRNA using RPC 5 absorbent (Miles Laboratories) have been described¹¹.

RPC-5 chromatographic profiles of AMV, RSV (Pra), and RSV (SR), 70S-associated RNA charged with methionine are qualitatively similar to the profiles shown by free viral Met-tRNA, chick embryo and myeloblast Met-tRNA (Fig. 1). In each case there are four peaks present and co-chromatography using ^3H and ^{35}S double labels shows that the corresponding species are chromatographically identical (data not shown). Quantitatively, there are distinct differences. Viral free tRNA that is not associated with 70S RNA shows a predominance of only one species, peak IV, which resembles chick embryo peak IV with respect to both structure and function. Peak IV is an m type of tRNA^{Met}, and donates its methionyl residue into internal positions of polypeptides synthesised *in vitro*¹¹. In contrast, viral Met-tRNA that is part of the 70S aggregate contains, in addition to peak IV, significant amounts of peak I,

Table 1 Incorporation of ^{35}S -methionine from AMV Met-tRNA species in response to AUG(U)₃₃

	^{35}S -methionine incorporated (c.p.m.)	
	No additions	20 μg AUG(U) ₃₃
70S-Associated peak I	834	6,661
70S-Associated peak IV	1,480	967
Free peak IV	1,211	1,459

Protein synthesis reactions were performed in a cell free system derived from mouse ascites cells^{11,14} in a total volume of 25 μl containing 40,000 c.p.m. of the appropriate ^{35}S -Met-tRNA. The Mg^{2+} concentration was 5 mM, the K^{+} concentration 80 mM; incubation was for 20 min at 37°C.

which in chick and mouse cells functions as the initiator tRNA¹¹. 70S-associated Met-tRNA also contains a lesser amount of peak III. A similar chromatographic profile of 70S-associated Met-tRNA was obtained with each of the viruses tested (AMV, RSV(Pra) and RSV(SR)).

The two classes of eukaryotic Met-tRNA have different nucleotide sequences near the amino acid acceptor end and digestion with ribonuclease T₁ liberates methionyl oligonucleotides that can be separated on electrophoresis at pH 3.5^{10,11}. In experiments not shown here, the terminal oligonucleotides of 70S-associated Met-tRNA I-IV were examined. Peak I oligonucleotide co-migrates with the corresponding oligonucleotide from ascites and chick cell Met-tRNA_f. Peak II contains both f and m terminal oligonucleotides, and peaks III and IV have a 3' OH terminus identical to ascites and chick cell Met-tRNA_m.

As mentioned above, one of the distinguishing features of the initiator tRNA is its ability to accept a formyl group. Although transformylase activity is not present in the cytoplasm of eukaryotes, Met-tRNA_f from these cells can react with *E. coli* transformylase to give fMet-tRNA_f^{10,12}. This can then be identified by the electrophoretic mobility of the terminal formyl-methionyl-adenosine, which is liberated on digestion with pancreatic ribonuclease. Figure 2 shows that when 70S-associated Met-tRNAs I-IV are treated in this manner only peak I (and peak I material which is a contaminant of peak II) is formylated. This species is therefore related to eukaryotic and bacterial initiator tRNA.

Function of 70S-associated Met-RNAs

The coding properties of tRNA^{Met}_f and tRNA^{Met}_m differ in that tRNA^{Met}_m recognises only internal AUG codons, whereas the initiator responds to either AUG or GUG at or near the 5' end of synthetic messengers^{10,13}. This difference can be detected by measuring the incorporation of methionine from the different species in response to synthetic mRNAs. The activity of viral 70S-associated Met-tRNAs in response to AUG(U)₃₃ was tested in a mouse ascites cell-free system. The results in Table 1 show that peak I responds markedly to this polymer and demonstrate its ability to function as an initiator tRNA *in vitro*. Peaks IV from both 70S-associated and free viral Met-tRNA are much less active, as is usual for tRNA^{Met}_m species^{10,13}.

In eukaryotes, all nascent polypeptides are thought to contain an N-terminal methionyl residue, donated by the initiator tRNA. In most cases, this methionine is subsequently cleaved from the growing peptide chain, presumably by a methionine amino-peptidase^{10,14}. Cleavage can be inhibited by preventing the nascent peptide from becoming sufficiently long to be a substrate for the cleavage enzyme. This can be achieved by use of the antibiotic sparsomycin which inhibits chain elongation but has little effect on chain initiation¹⁵. Thus, in the presence of the antibiotic short chain initiation peptides accumulate which are labelled with methionine exclusively from Met-tRNA_f. Figure 3 shows an autoradiograph of peptides made in the presence of sparsomycin and separated on electrophoresis at pH 3.5. 70S-associated peak I donates methionine into the

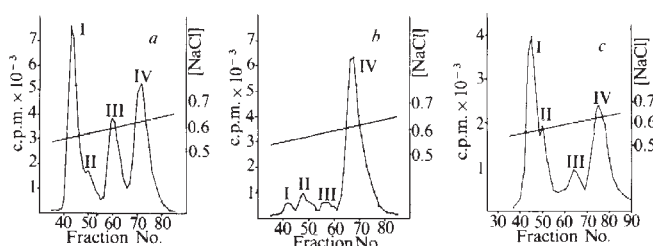


Fig. 1 RPC-5 chromatography of ^{35}S -Met-tRNAs from, a, chick embryo; b, AMV-free 4S RNA; c, AMV 70S-associated 4S RNA. 10^5 c.p.m. of each aminoacyl-tRNA were applied to a 0.9×30 cm column of RPC-5. The isoaccepting species were eluted with a linear gradient of 0.5–0.7 M NaCl (total volume 100 ml) in 10 mM NaAc, 10 mM MgCl_2 , 2 mM 2-mercapto-ethanol at pH 4.5. The flow rate was 60 ml h⁻¹ and 0.75 ml fractions were collected and counted in a scintillation fluid containing PPO, POPOP, and Triton X100 (refs 11, 20).

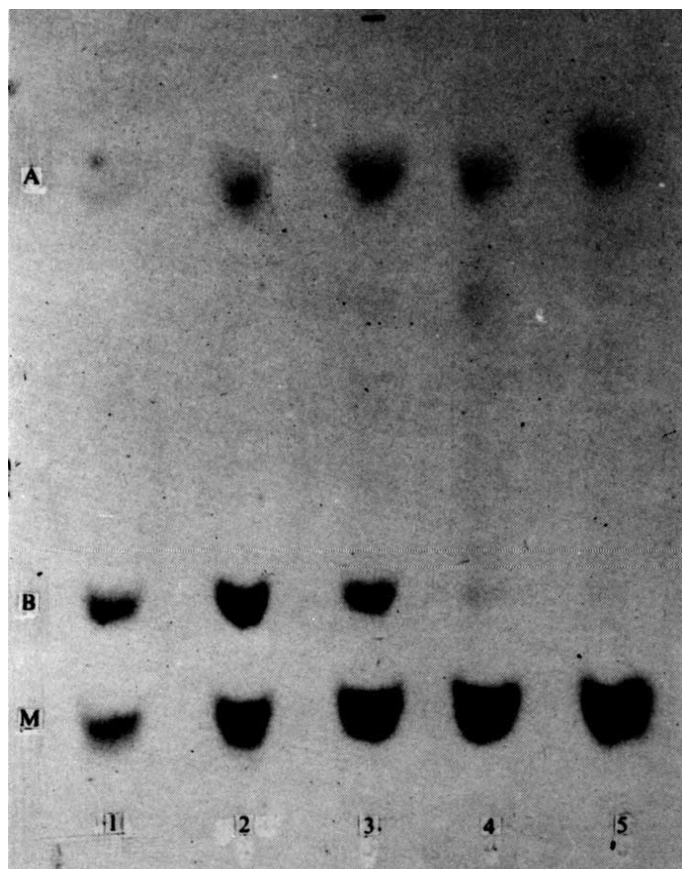


Fig. 2 Pancreatic ribonuclease digest of separated ^{35}S -Met-tRNAs after reaction with *E. coli* transformylase and formyl donor. Formylation reactions (25 μl) were carried out in an incubation mixture containing 100 mM Tris HCl, pH 7.5, 10 mM MgCl_2 , 10 mM KCl, 6 mM ATP, 3 mM CTP, 6 mM 2-mercaptoethanol, 0.5 mg ml^{-1} *E. coli* supernatant fraction (which had been concentrated by ammonium sulphate precipitation), 0.25 mM calcium leucovorin and 10,000 c.p.m. of purified peaks I-IV isolated from AMV 70S RNA. Incubation was continued for 5 min at 37°C , one drop of pancreatic ribonuclease A (200 $\mu\text{g ml}^{-1}$ in H_2O) added, and the mixture immediately applied to Whatman 3 mm paper for electrophoresis at pH 3.5 (60 V cm^{-1} , 30 min). The dried paper was autoradiographed for 2 weeks with Osray M film. 1, Chick peak I; 2, AMV 70S associated peak I; 3, peak II; 4, peak III; 5, peak IV. A, methionyl adenosine; B, formyl-methionyl adenosine; M, free methionine. Note that during incubation at 37°C , those species of Met-tRNA that are not enzymatically formylated are to some extent chemically deacylated and give free methionine rather than methionyl adenosine.

specific initiation peptides made in response to both encephalomyocarditis virus RNA (EMC RNA) and AUG(U) $_{35}$. Free viral peak IV, on the other hand, is inactive in labelling sparsomycin peptides.

The experiments described above show that 70S-associated Met-tRNA peak I is related to normal chick initiator tRNA in both structure and ability to take part in protein synthesis. In experiments similar to those already reported for free viral Met-tRNA peak IV, we have confirmed that 70S-associated peak IV is active in donating methionine into polypeptide synthesised in response to EMC RNA (Table 2).

Other experiments which will be published in detail elsewhere have shown that the polypeptide synthesised in response to EMC RNA and labelled with methionine from different Met-tRNA $_m$ species, is identical no matter whether the tRNA used was from mouse or chick, or free AMV or 70S RNA-associated fractions. Furthermore we can detect no difference in the peptide labelling pattern obtained using Met-tRNA from either peak III or peak IV.

Wang, Kothari, Taylor and Hung (personal communication)

Table 2 Incorporation of ^{35}S -methionine from chick and AMV Met-tRNA species in response to EMC RNA

	^{35}S -methionine incorporated (c.p.m.)	
	No additions	Plus 2 μg EMC RNA
Chick peak I	522	1,233
Chick peak III	2,042	9,236
Chick peak IV	2,136	11,028
AMV free peak IV	2,059	10,210
AMV 70S associated IV	1,567	7,921

Incubation conditions were exactly as described in Table 1, except that the Mg^{2+} concentration was 3 mM and the K^+ concentration 120 mM.

have also shown that the free AMV Met-tRNA of peak IV is not an initiator species.

Role of viral tRNAs

tRNA $^{\text{Met}}$ is required for the initial step in protein synthesis. There are many properties of the eukaryotic initiator tRNA that distinguish it from normal tRNAs, including the reaction with bacterial methionyl-tRNA synthetase¹⁶, and transformylase¹², the recognition of initiation factors¹⁷, the inability to form a stable complex with elongation factors¹⁸, and the unusual

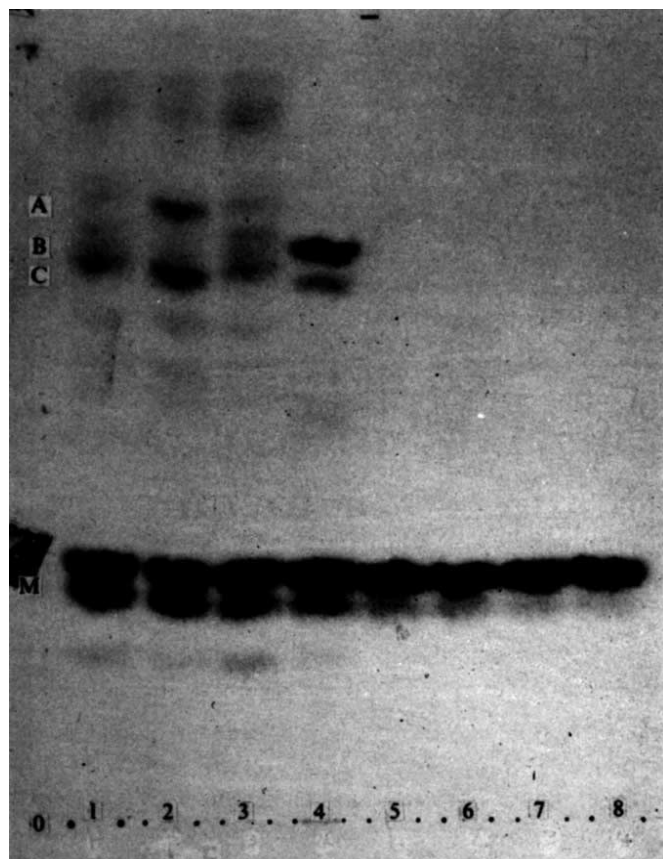


Fig. 3 Sparsomycin peptides labelled with methionine from AMV Met-tRNAs. A detailed description of the sparsomycin assay has been published¹⁵. Briefly, cell-free reactions were performed in a total volume of 50 μl , containing 66 μM sparsomycin. After 10 min incubation at 37°C the sparsomycin peptides were isolated, separated by electrophoresis at pH 3.5 (60 V cm^{-1} , 75 min) and visualised by autoradiography (10 d, Osray M film). Incubations 2 and 6 included 3 μg EMC RNA, 4 and 8 40 μg AUG(U) $_{35}$, 1-4 contained 50,000 c.p.m. AMV 70S-associated Peak I, Met tRNA; 5-8 55,000 c.p.m. AMV free peak IV Met-tRNA. 1, 2, 5 and 6 were at 3 mM Mg^{2+} and 120 mM K^+ ; 3, 4, 7 and 8 at 5 mM Mg^{2+} and 80 mM K^+ ; all other ingredients were as published^{11,15}. A, Met Ala; B, Met Phe; C, Met Ala Thr; M, free methionine and methionine sulphoxide; O, origin.

coding properties¹³. All these properties strongly suggest that the unique structure of tRNA^{Met} has been specially adapted for its role in the initiation of protein synthesis. The presence of this unusual molecule in close association with tumour virus nucleic acid is therefore somewhat surprising. 4S RNA molecules are required as primers for reverse transcriptase which synthesises viral DNA¹⁹. Although viral tRNA^{Met} seems to be identical to the host cell molecule both in its structure and its ability to function as an initiator tRNA, it is possible that tRNA^{Met} is also involved as a primer in the transcription process. It is equally possible that another of the 70S-associated 4S RNA molecules, including the other methionine accepting species, might be involved as a primer. The role of those tRNA species that are not primer molecules is unclear, but they may be necessary to maintain the structure of the genome, or be an example of tRNA modulation²¹. A definite answer to these questions must await further analysis of the structure of the primer molecule and the role of virus-associated tRNAs in the translation of tumour virus messenger RNA.

Contaminating degradation products of the high molecular weight RNA may also be released by denaturation of 70S RNA. Hence, it is difficult to calculate the number of molecules of tRNA^{Met} associated with the 70S RNA. A crude estimate indicates that there may be about one mol of methionine acceptor activity per mol of 70S RNA. Since the amount of free 4S RNA in different virus preparations is variable, a calculation of the amount of free tRNA^{Met} has little meaning. In general, however, there is usually five to ten times more tRNA^{Met} free in the virion than associated with 70S RNA. The significance of the distribution of tRNAs between viral compartments is, as yet, unexplained.

The origin of the viral tRNAs is also uncertain but the similarity in structure and function between host and both free and associated viral methionine accepting tRNAs strongly suggest that the viral tRNAs are of host cell origin and are not virus coded.

We thank Dr R. L. Erikson and Dr T. Walker for providing RSV and AMV 70S RNA, and Dr J. C. Brown for AUG(U)₃₃. We thank Tricia Wheeler for technical assistance, Dr Milton Taylor for allowing us to see a manuscript prior to publication and Dr R. L. Erikson for advice. This work was supported by the Imperial Cancer Research Fund and US Public Health Service grants.

Received October 30, 1973.

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Lateral diffusion of rhodopsin in the photoreceptor membrane

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Rhodopsin undergoes rapid lateral diffusion in the disk membranes of isolated frog and mudpuppy rods. The rate of lateral diffusion is consistent with the rapid rotational diffusion of rhodopsin: both indicate the disk membrane is highly fluid with a viscosity of ~1P.

HIGH-speed flash photometry has shown that rhodopsin undergoes rapid rotational diffusion in the disk membrane of frog rod outer segments¹. Rotational diffusion about an axis perpendicular to the membrane occurs with a relaxation time about 20 μ s at 20°C. Rhodopsin is deeply embedded in the membrane, and this relaxation time indicates that the site it occupies is highly fluid, with an effective viscosity for rotational diffusion comparable with that of a light oil. Rhodopsin molecules appear from X-ray evidence to be distributed in a liquid-like array in the plane of the membrane², so they may also be free to undergo rapid lateral diffusion in the

membrane. Recently, evidence has been reported for several types of cells that membrane proteins can undergo rapid lateral motion³⁻⁶, and it is clear that a general understanding of molecular events within cell membranes will require more knowledge of the diffusional motions of membrane proteins. We report here microspectrophotometric observations of rapid lateral diffusion of rhodopsin in freshly isolated rod outer segments.

Rod outer segments were obtained by gently shaking retinas dissected under dim red light from the eyes of frog (*Rana catesbeiana*) and mudpuppy (*Necturus maculosus*) which had been dark-adapted for more than 10 h. The rods were shaken into a microchamber containing a standard Ringer solution and examined in a Shimadzu 50L microspectrophotometer (MSP) fitted with a high quantum efficiency photomultiplier (Hamamatsu type R375). Single rods which appeared intact and which lay flat on the bottom of the chamber were selected for observation, and all observa-

tions were completed within 30 min after the rods were isolated. The rhodopsin in isolated rods, once bleached, does not regenerate, hence dim red light was used for selection, focusing, and alignment.

The measuring beam of the MSP was limited by an aperture and a condensing lens to form a rectangle about $2 \times 20 \mu\text{m}$ in cross section. The long axis of the rectangle was aligned with the long axis of the rod and a simple motor-driven 'alternator' optically shifted the rectangular measuring beam back and forth between the two sides of the rod. Thus the absorbance of the rhodopsin on each side of the rod could be compared directly. The wavelength of the measuring beam was set at the absorption peak of the visual pigment: 500 nm for frog, 530 nm for mudpuppy.

With suitable alignment and focusing the absorbance was essentially equal on both sides of unbleached rods, as shown by the first pair of measurements on an unbleached rod at the beginning of each of the two recordings in Fig. 1. The alternator was then stopped momentarily and the intensity of the measuring beam was increased about 1,000-fold to bleach some pigment on one side of the rod. The exponential decrease in absorbance during the bleach was recorded, and then the intensity was dropped to the original level and the alternator turned on again. Figure 1 shows that immediately after the bleach the absorbance on the unbleached side was little changed, but there was a marked drop in absorbance on the bleached side. Within the next few seconds, however, the absorbance of the unbleached side decreased while that on the bleached side increased, and within less than 1 min the absorbance of the two sides became equal, reaching a final level midway between that of each side immediately after the bleach. This is expected if rhodopsin molecules can diffuse laterally in the membrane: the total pigment present after the bleach remained constant and only its distribution changed, approaching uniform distribution with an exponential time course. The half-time of the final approach to uniform distribution has been observed in more than ten rods each for frog, and mudpuppy. At 20°C , it was $35 \pm 7 \text{ s}$ for frog rods $8 \mu\text{m}$ in diameter, and for mudpuppy rods $23 \pm 5 \text{ s}$ for rods $12 \mu\text{m}$ in diameter. In addition, half-times measured at temperatures in the range $17^\circ\text{--}24^\circ\text{C}$ decreased with temperature with a Q_{10} of 3.7 ± 0.7 in frog rods and 3.1 ± 1.4 in mudpuppy rods (apparent energy of activation 24 and 21 kcal/mol respectively).

Control experiments were performed to help ensure that the observed redistribution of rhodopsin was the result of diffusion of the entire rhodopsin molecule and not simply energy transfer between chromophores or chromophore transfer between protein molecules (opsin). As Fig. 2 shows, there was no redistribution of rhodopsin in rods fixed with glutaraldehyde before bleaching. In contrast, fixation with the same amount of formaldehyde had no effect on the rate of redistribution. These results are consistent with the findings that glutaraldehyde fixation blocks rotational diffusion of rhodopsin whereas formaldehyde fixation has no effect^{1,7}. Glutaraldehyde seems to form crosslinks between rhodopsin molecules whereas formaldehyde does not, for rhodopsin can be extracted with digitonin after formaldehyde fixation but cannot be extracted after glutaraldehyde fixation⁷. Chromophore transfer between opsin molecules seems unlikely given the nature of the covalent linkage between retinal and opsin, but as a further check experiments were performed in the presence of 50 mM hydroxylamine which rapidly converts free chromophore to the stable compound retinal oxime ($\lambda_{\text{max}} = 363 \text{ nm}$)⁸. When one side of the rod was bleached, all chromophores from the bleached pigment converted immediately to retinal oxime. The redistribution of rhodopsin, however, proceeded as usual, no further oxime was formed and the total remaining pigment stayed constant. Thus the chromophores of the unbleached pigment did not react with hydroxylamine, as might be expected if transfer of free

chromophores accounted for the redistribution of pigment. Energy transfer between rhodopsin molecules is also improbable since the distance between chromophores is large^{9,10}, and the fluorescence yield is low¹¹. Moreover, energy transfer cannot explain the redistribution of pigment since pre-lumi rhodopsin, the first photoproduct after bleaching, forms within a few picoseconds at room temperatures¹². Thus energy transfer between rhodopsin molecules, if it occurs, would have to take place on a sub-picosecond scale, whereas the observed redistribution takes place in seconds. Finally, as a further test for the plausibility of energy or chromophore transfer, we investigated whether redistribution of pigment occurs along the axis of the rod. Rods were bleached with a black and white 'grating' pattern which alternated in intensity along the axis of the rod with a repeat distance of $8 \mu\text{m}$. Rhodopsin absorbance was monitored by scanning the length of the rod with the measuring beam in this case being limited to a circular spot $4 \mu\text{m}$ in diameter. After the bleach the resulting 'grating' pattern in absorbance persisted unchanged for as long as stable recordings could be made ($\sim 10 \text{ min}$)¹³. Thus the redistribution of rhodopsin seems to be confined to the plane of the disk membrane, since no migration along the length of the rod was detected. This result is consistent with the autoradiographic investigations reported by Young and his co-workers¹⁴ which show that once rhodopsin is inserted into the membrane of a disk, it does not migrate away from that disk. From all these considerations, lateral diffusion of entire rhodopsin molecules within the disk membrane seems to be the only reasonable explanation for the redistribution of pigment shown in Fig. 1.

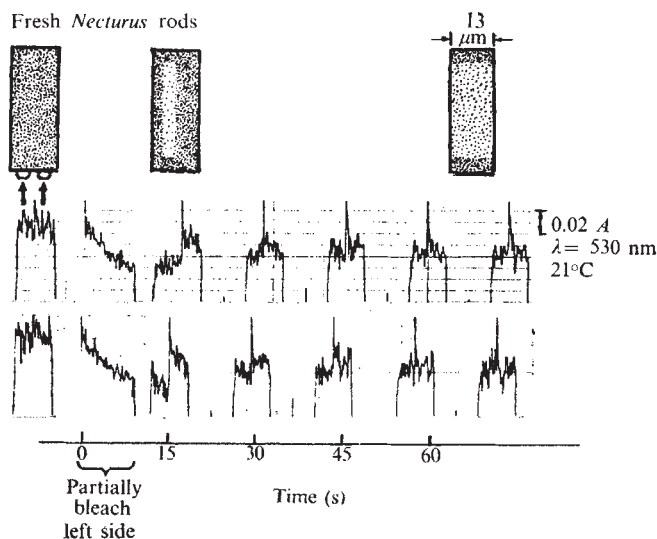


FIG. 1 Lateral diffusion of rhodopsin in the disk membrane. The diagrams of a rod depict the pigment distribution corresponding in time with the absorbance measurements shown below. The arrows indicate the location on the rod at which each absorbance measurement was made. Recordings made from two different rods are shown to give an indication of the repeatability of the measurements. In each experiment the chart recorder was run continuously, as shown by the time base. The alternator also ran continuously except during the bleach. The records thus consist of a repeated pattern in which absorbance measurements were made first on the left side, then the right side of the rod. Between each pair of measurements baseline measurements were also made to ensure that no drifts occurred (for clarity, these were omitted from the figure). The spikes on the traces were caused by switching transients in the alternator. The diameter of a disk is essentially equal to the width of the rod, and the width was measured in the MSP after completing each experiment. In a separate experiment described in the text¹³, it has been shown that no diffusion of rhodopsin occurs along the length of the rod. Thus in the diagrams of a rod, the rhodopsin concentration is depicted as unchanged at the ends, where no bleaching occurred.

The half-time for redistribution of rhodopsin did not seem to depend on the amount of uniform bleaching which in some cases occurred before starting the diffusion experiments. Redistribution proceeded as usual even when two-thirds of the pigment was previously bleached. Since rhodopsin molecules occupy a major fraction ($\sim 1/3$ to $2/3$) of the total membrane area, redistribution can only occur if rhodopsin molecules displace bleached molecules (opsins). Thus opsin molecules appear to have essentially the same diffusion constant.

The diffusion constant, D , for lateral diffusion of rhodopsin in the disk membrane can be obtained by solving the two-dimensional diffusion equation for the closed boundary defined by the edge of the disk. The result is, to first order of accuracy,

$$D = (0.69bL^2)/(\pi^2 t_{1/2}) \quad (1)$$

where L is the diameter of the disk $t_{1/2}$ is the half-time with which pigment distribution approaches final uniform distribution, and b is a geometry factor included to account for the irregular boundary of the disk, especially the fissures which extend well in toward the centre. To obtain the value for b , we performed an analogue experiment in which the rate of diffusion of heat was observed in thin sheet-metal disks with the boundary cut to simulate the shape of typical disks in frog and mudpuppy rods as observed by electron microscopy¹⁵. The value obtained was 2.7 ± 1.0 for frog disks which have numerous deep fissures, and 0.9 ± 0.2 for mudpuppy disks which have numerous but very shallow fissures. For these values of b , and the observed values of L and $t_{1/2}$, equation (1) yields

$$D = (3.5 \pm 1.5) \times 10^{-9} \text{ cm}^2 \text{ s}^{-1} \quad \text{for frogs}$$

$$D = (3.9 \pm 1.2) \times 10^{-9} \text{ cm}^2 \text{ s}^{-1} \quad \text{for mudpuppy}$$

Viscosity of membrane

The diffusion constant is a measure of diffusion rate, depending not only on the viscosity of the medium in which diffusion occurs, but also on the size of the diffusing particle. Therefore, even within a given medium diffusion constants for various particles cannot be compared directly. However, as Einstein showed, the viscosity of a solution can be predicted by observing the diffusion constant of a large solute molecule in the solution and by assuming that in undergoing thermal motions the solute molecule experiences a viscous drag given by Stoke's law¹⁶. Later theoretical treatments have shown that only a minor numerical correction is needed to extend this procedure to molecules with dimensions comparable with those of the solvent molecules¹⁷. Thus, although viscosity is usually observed macroscopically, it is a property of solutions which can be observed equally well using small molecules, even molecules as small as the solvent molecules. In membranes, diffusion constants have been observed for various probes using different techniques. It seems reasonable to expect that regardless of the size and shape of a probe molecule or structure, as long as a significant portion of it spans a major fraction of the membrane thickness, its rate of diffusion in the plane of the membrane, when properly interpreted, should yield the same viscosity. Hence diffusion constants for different probes can be compared by comparing the predicted viscosities.

The rotational diffusion of rhodopsin, as previously reported¹, indicates the viscosity, η , of the membrane site occupied by frog rhodopsin is about 2 P at 20°C. The calculation was based on the relationship derived by Einstein for Brownian rotation of a spherical particle about one axis in a isotropic medium. A similar calculation can be made for lateral diffusion by using the Einstein-Sutherland diffusion equation¹⁷

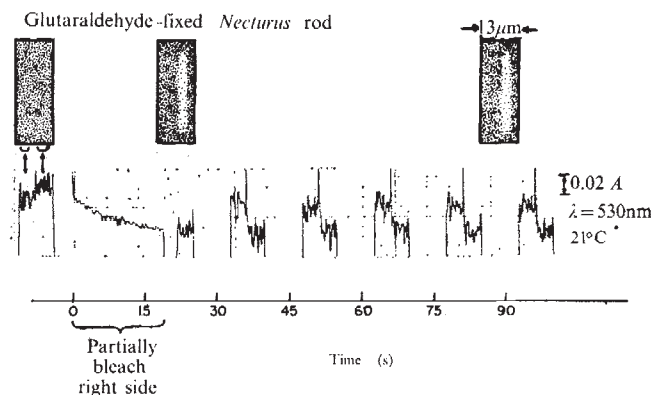


Fig. 2 Glutaraldehyde fixation blocks lateral diffusion of rhodopsin in rod disks. Isolated mudpuppy rod outer segments were soaked in 1% glutaraldehyde in 0.1 M phosphate buffer (pH 7.1) for 15 min before starting the experiment. The diagrams again depict the distribution of pigment at the time of measurements shown below. Although the recording procedures were the same as described in Fig. 1, after glutaraldehyde fixation the unequal distribution produced by the bleaching persisted as long as stable recordings could be made. It may be noted in this experiment that stray light caused negligible bleaching on the unbleached side. Hence in the first recordings after the bleach in Fig. 1, the small decrease in absorbance on the unbleached side is the result of some diffusion having already occurred.

$$\eta = (kT)/(a\pi Dr) \quad (2)$$

where k is the Boltzman constant, T is the absolute temperature, r is the radius of a spherical diffusant, and $a = 6$ for diffusants large compared with the solvent molecules, while $a = 4$ for diffusants with dimensions comparable with the solvent molecules. Recent evidence indicates that the rhodopsin molecule spans the membrane^{18,19} (see also discussion of ref. 13) and on the basis of molecular weight²⁰ and X-ray evidence⁹, the effective radius of rhodopsin for lateral diffusion is probably in the range 15–30 Å. This is significantly larger than the effective radius of phospholipids for lateral diffusion. In this case equation (2) yields a viscosity of about 1–4 P. This is in the same range indicated by the rotational diffusion of rhodopsin, 0.7–6 P¹. Thus if it is assumed that rhodopsin has an effectively circular cross section, the viscosity of the disk membrane indicated by lateral diffusion is in good agreement with the viscosity indicated by rotational diffusion. This is not surprising since lateral diffusion in the membrane and rotational diffusion about an axis perpendicular to the membrane surface both depend on the same 'slippage' motion between the surface of the rhodopsin molecule and the molecules in the surrounding membrane environment. Moreover, as we show when, each rhodopsin molecule undergoes numerous collisions with other rhodopsin molecules during its rotational relaxation time. Thus a rhodopsin molecule samples essentially the same membrane environment for both its rotational and translational motions.

Evidence that other membrane proteins may also exist in such highly fluid environments has been reported by Frye and Edidin who observed, using fluorescent antibodies, the translational diffusion of cell surface antigens in membranes of mouse and human cell hybrids³. The rate at which the surface antigens diffuse along the cell surface indicates the membrane viscosity for lateral diffusion of these proteins is on the order of 1–10 P at 20°C. (For a recent discussion of membrane fluidity, see ref. 21.) As pointed out previously¹, lipid soluble spin labels seems to encounter a viscosity of 0.1–1 P at 20°C in the lipid phase of mitochondria and nerve axons^{22,23}. Spin label studies by Scandella *et al.* on lateral diffusion of phospholipids in sarcoplasmic reticulum

vesicles from rabbit muscle²⁴ have shown the diffusion constant to be about $6 \times 10^{-8} \text{ cm}^2 \text{ s}^{-1}$ at 40°C. Using the Einstein-Sutherland equation for self-diffusion, a reasonable extrapolation of this result to 20°C indicates that membrane viscosity falls within 0.5–5 P. Thus in spite of differences in probes and techniques the viscosity of each of these membranes seem to lie within an order of magnitude of 1 P. That is, the viscosity for lateral motion in each membrane is between 10 and 1,000 times the viscosity of water. Differences in the composition of each membrane and in the membrane layer sampled by the probe molecule, together with possible systematic experimental errors, can account for the variations in viscosity so far observed. Rhodopsin can now be incorporated into model membrane systems^{25–27} with which it should be possible to study quantitatively the effects of each membrane component on membrane viscosity.

Rhodopsin collision frequency

X-ray evidence indicates that the average nearest-neighbour distance between rhodopsin molecules in the disk membrane is about 70 Å (refs 9 and 10). The average time τ , between collisions with another rhodopsin can be estimated with the following equation in which s is the distance rhodopsin travels between collisions:

$$s^2 = 4D\tau.$$

If the effective diameter of rhodopsin is about 45 Å (ref. 9), then s is 25 Å, and the average time between collisions is about 4 μs at 20°C. This is considerably less than the 20 μs relaxation time for rotational diffusion of rhodopsin, and indicates that the collision frequency between rhodopsin molecules is in the range 10^5 – 10^6 collisions per second.

The rapidity of rhodopsin-rhodopsin collisions points out an important consequence of the highly fluid nature of cell membranes, namely, the potential rapidity of reactions between membrane proteins. By partitioning into the membrane, protein molecules may achieve much higher effective concentrations and can in addition maintain favourable orientations as a result of protein-lipid interactions. Thus, since the viscosity for lateral motion in the membrane exceeds that of water by only a factor of 10–1,000, reaction rates both

within and on the surface of a membrane may potentially exceed by many orders of magnitude the rates which would occur if the reactants were dispersed throughout the aqueous phase.

We thank Dan Petersen for help with the manuscript, and Paul K. Brown for electron micrographs of *Necturus* rod disks.

This work was supported in part by a grant from the US National Institutes of Health.

Received October 9; revised November 29, 1973.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Limits on variation of G from clusters of galaxies

If G decreases by a sufficient amount a cluster of particles will expand. In some cases the particles may have sufficient velocity to escape from the cluster as the gravitational binding decreases. Since it is known that clusters of galaxies and globular clusters currently exist with finite dimensions, limits can be set on the magnitude of the variation of G . In order to set a limit on the rate of change of the gravitational constant ($\dot{G}/G$), the dynamical effects on an isolated cluster of galaxies caused by non zero values of $\dot{G}/G$ were studied numerically. For a range of initial conditions, the maximum

rate of change in G was determined which would still allow the existence of clusters of galaxies at the current epoch. A similar study was made for globular clusters and the results were found to be comparable.

The gravitational fields of both the cluster of galaxies and the globular cluster were simulated by Plummer potentials¹, and the effect on a particle (galaxy/star) of various rates of G variation were observed. The distribution of galaxies in a cluster at the time of formation is unknown, but if gravitational forces were dominant, a Plummer potential should provide a reasonable approximation to the cluster's field. The Plummer potential was initially designed to simulate the potential field of a globular cluster, and has the form

$$\Psi = \Psi_0/[1 + (r/r_0)^2]^{1/2}$$

where $\Psi_0 = M_0 G/r_0$ and r_0 and M_0 are chosen using a method given by Henon². We assumed that both the globular

cluster and the cluster of galaxies were initially stable (twice the kinetic energy, T , plus the gravitational energy, Ω , is zero). For our standard cluster of galaxies we chose $M = 2.5 \times 10^{13} M_\odot$ and $r_0 = 0.17$ Mpc. This is about the mass of a Virgo type cluster, and r_0 was chosen a bit small to allow for expansion. It was demonstrated by Dearborn³ that the resultant potential yields results consistent with those of an N -body calculation. Our standard globular cluster has $M = 10^6 M_\odot$ and $r_0 = 1.33$ pc. In reality, as the cluster expands due to a decreasing G , r_0 increases and $2T + \Omega$ becomes greater than zero. The shape of the Plummer potential would then change to a less stable configuration. In our calculation r_0 was held constant and $2T + \Omega$ kept equal to zero. Therefore, the potential was forced into a more stable shape than it would actually have, thus the limit obtained on G/G is even stronger than if we tried to vary r_0 for the potential with time.

The calculations were further simplified by using radial orbits for most of the work. For completeness, we did study several cases with circular orbits for both clusters of galaxies and globular clusters. Due to angular momentum effects, the expansion of circular orbits was even greater than that of radial orbits. Since orbits in real clusters are intermediate cases, the use of radial orbits gives the strongest limit.

For most of our calculations we used a form for G/G of

$$(\dot{G}/G) = -\alpha/t \quad \text{or} \quad G = G_i(t_i/t)^\alpha$$

when G_i is the value of G at time t_i .

This form is of particular interest, because it describes a change in G predicted by the Brans-Dicke theory. (The Dirac cosmology also predicts this form for G/G but with $\alpha = 1$). The t_i in this form is the time of the initial formation of galaxies in the cluster. This is not necessarily the time at which stars formed, but the time at which galaxy size masses condensed out of the intergalactic medium. Since such a time is open to speculations, we considered a range of initial times including 1.6×10^7 yr, 10^8 yr, and 10^9 yr. We feel, however, that the formation time given by Partridge and Peebles⁴ of 2×10^8 yr (for a constant G) is reasonable. In fact, it seems reasonable that if G were initially larger, the speed of formation would be greater and t_i even smaller (B. Tinsley, private communication). For each of these initial times it was then possible to determine a limiting coefficient, α_{lim} , for which galaxies beginning near the centre of the potential attained escape velocity in a time less than the age of the universe.

Using relations given by Weinberg⁵ it is possible to determine a limit on ω (the dimensionless coupling parameter in the Brans-Dicke Theory) for a given value of the cosmological deceleration parameter, q_0 , and the co-efficient α . The relations are:

$$\alpha/t_0 = 3q_0 H_0 / (2 + \omega) \quad \text{for} \quad q_0 \ll 1$$

$$\text{and} \quad \alpha/t_0 = H_0 / (2 + \omega) \quad \text{for} \quad q_0 = 1/2$$

The present estimated value for q_0 is 1 ± 1 (ref. 6) but a recent study by Gott and Gunn⁷ on the effects of infalling matter on a cluster of galaxies indicates $q_0 \ll 0.1$. Further arguments for a small q_0 are given from the evolution of Galaxies by Tinsley⁸ and from cosmological deuterium production⁹.

We also consider the form

$$\dot{G}/G = -1/\tau \quad \text{or} \quad G = G_i \exp(-(t - t_i)/\tau)$$

This exponential form for the variation of G/G is predicted by no theory known to us and is examined merely out of curiosity.

In Fig. 1 the maximum value of α (α_{lim}) consistent with the existence of clusters of galaxies is given for different initial times (t_i) and starting positions (R) in the cluster.

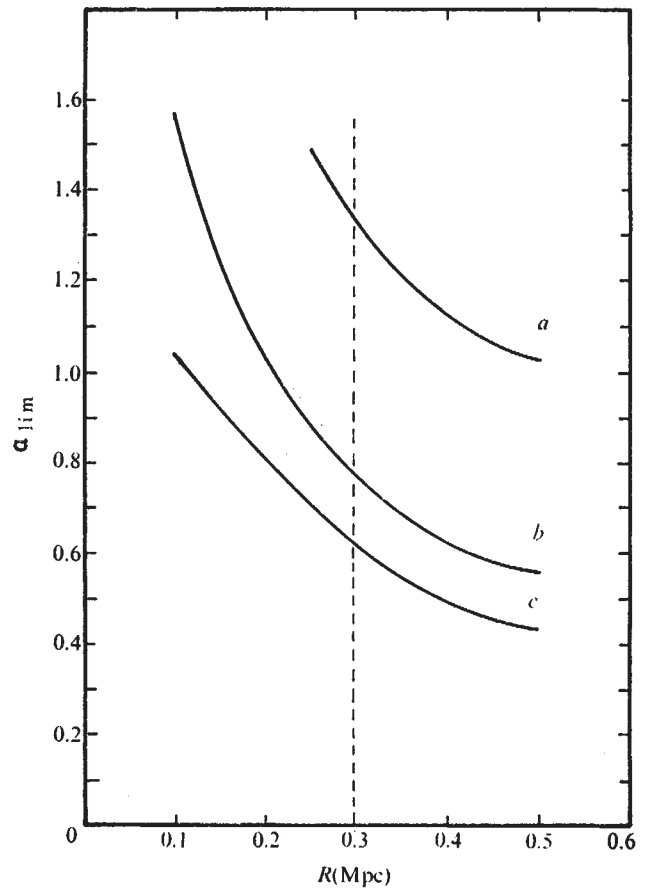


Fig. 1 Limiting α consistent with the existence of clusters at the current epoch for various initial radii, R and times t_i . Values of α_{lim} enable galaxies to escape in times less than the age of the universe. For $R < R_{crit}$ the Plummer potential is no longer accurate and α_{lim} is approximately constant due to the effects of close interactions. In our case R_{crit} is ~ 0.3 Mpc. Since α_{lim} increases for decreasing R the upper bound to α_{lim} is obtained at $R = R_{crit}$. $G/G_0 = -\alpha/t_0$. a, $t_i = 10^9$; b, $t_i = 10^8$; c, $t_i = 1.6 \times 10^7$.

The limiting values of α were determined for a given t_i and R by evolving a case with several different α s. It was then possible to see at what value of α particles would escape in less than the age of the universe (escape was usually in less than 7×10^9 yr).

The initial position (R) of particles in the potential effects the limit that can be set on G/G . In agreement with N -body calculations³, particles near the centre of the potential need a higher G/G to escape than do particles near the edge. But unlike the N -body solution, a particle at rest at the centre of a potential never escapes. In an N -body solution, such a particle would be perturbed into motion and escape along with other particles near the centre. It is therefore necessary to realize that the α_{lim} curves given in Fig. 1 (determined with a Plummer potential) should actually approach a finite value instead of going to infinity as the starting radius approaches zero.

It is possible to estimate a radius, R_{crit} , within which close interaction perturbations are significant relative to the background potential of the cluster. For initial radii, R , less than some radius, R_{crit} , the orbit of a particle in a Plummer potential is no longer a valid representation of the motion of a particle. Particles with $R < R_{crit}$ could be perturbed outward by close interactions, therefore the true value of α_{lim} does not increase significantly for $R < R_{crit}$. For an initial radius R less than $2r_0$ (0.34 Mpc in our case), the Plummer potential is probably no longer an accurate approximation to reality. Orbits for particles with initial radii ranging from

0.1 to 0.5 Mpc were studied, but we believe a value of $R_{crit} = 0.3$ Mpc is a good lower bound to possible values. Since α_{lim} increases as R decreases the upper limit to α_{lim} occurs at $R = R_{crit}$.

Using $t_i = 2 \times 10^8$ yr and $R = 0.3$ Mpc, it is obvious from Fig. 1 that $\alpha_{lim} \cong 0.8$. This corresponds to values of $\dot{G}/G$ of

$$\begin{aligned}\dot{G}/G &= -\alpha/t_0 = 4 \times 10^{-11} \text{ yr}^{-1} \text{ for } q_0 \ll 1 \\ &= 6 \times 10^{-11} \text{ yr}^{-1} \text{ for } q_0 \\ &= 1/2 \text{ with } H_0 = 55 \text{ km s}^{-1} \text{ Mpc}^{-1}.\end{aligned}$$

Even stronger limits might be achieved if different values for t_i and R are assumed. But the above estimates can be stated with reasonable confidence. (For the exponential case the limit was 10^{-10} yr^{-1} .) These limits on $\dot{G}/G$ are much stronger than Shapiro *et al.*'s¹⁰ limit of $4 \times 10^{-10} \text{ yr}^{-1}$. But the limit on the Brans-Dicke ω determined from these limits on α_{lim} is still very weak and does not put any real constraints on the scalar component in the Brans-Dicke theory. Therefore, the Brans-Dicke scalar component is not inconsistent with the existence of clusters of galaxies. It can be said, however, that the Dirac Cosmology is not consistent with the present limit. Similarly the Hoyle-Narlikar Cosmology¹¹ with $\dot{G}/G \propto t^{-1}$ would seem inconsistent with the observations.

For the globular cluster case, a similar result can be obtained. But effects of $\dot{G}/G$ on a globular cluster will not be so dramatic because the crossing times are short relative to the time in which G changes significantly. The effect of $\alpha = 1$ on our standard globular cluster with $t_i = 1.6 \times 10^7$ yr was calculated. This t_i is appropriate for the time of formation of globular clusters⁴. After 1.5×10^{10} yr, a particle starting at $R = 1.5$ pc has an orbit 130 times larger. If the initial radius were reduced 130 times, the cluster evolves $\sim 1,500$ times faster and so would still expand to radii $\gg 1.5$ pc. Initially, the cluster would be highly relativistic. Normally relativistic clusters collapse on time scales of 10^8 to 10^9 yr (ref. 12) but it is possible that a decreasing G may allow a relativistic cluster to expand to become a non-relativistic cluster instead of collapsing. Even so, such a cluster would have experienced a large number of stellar collisions. Such an extreme initial star density for any currently observed globular cluster seems very unlikely. So the limit on the variation of G from the current existence of globular clusters is approximately the same as that obtained from the current existence of clusters of galaxies.

While this work was in progress Morrison¹³ proposed, by independent means, a limit of similar magnitude on $\dot{G}/G$. He used occultations timed on atomic time scales (IAT) to determine the slowing in the Earth's rotation due to tidal and nontidal ($\dot{G}/G$) forces. Previous determinations were based on data timed on a gravitational basis on which he assumed effects of $\dot{G}/G$ would not be apparent, leaving only tidal slowing. The close agreement of the decelerations rates then implies $\dot{G}/G = 0 \text{ yr}^{-1}$, yielding an upper limit on $\dot{G}/G$ from the uncertainty in the data which is approximately the same as the limit estimated in this work.

We thank W. D. Arnett, P. J. E. Peebles, D. Mulholland and B. Tinsley for comments. This research was supported in part by a National Science Foundation grant.

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Received October 24, revised December 3, 1973.

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Radio spectra for three QSOs

RADIO spectra are presented for three sources of current interest: 1548 + 114 (4C 1150) which is one of two quasars separated by about 5 arc s which have been suggested as possibly associated despite widely disparate redshifts¹; 1331 + 170 which is a very luminous QSO with a rich absorption spectrum and suggested line locking between the emission spectrum and two separate absorption systems²; and 1442 + 101 (OQ172) which has the currently highest known redshift³, $z = 3.53$.

All three sources occur in two sections of the Molonglo catalogue survey currently being prepared for publication (J. M. Sutton, I. M. Davies, A. G. Little and H. S. M., unpublished). These cover declination strips approximately 2° wide, centred on $+11.2^\circ$ and $+16.5^\circ$ declination. The sources in this survey are being studied in detail. Optical identifications have been carried out for a large fraction of the sources (C. Hazard and H. S. M., unpublished) and optical spectroscopic data on sixty-eight of the sources have been presented by Baldwin *et al.*⁴

The radio spectra are given in Fig. 1. The flux densities at 80 MHz and 178 MHz for 1548 + 114 have been increased by 20% and 15% respectively^{5,6}. The 606 MHz flux density was obtained at Arecibo and the 1,400 and 2,695 MHz flux densities have been obtained with the 300 foot telescope at Green Bank. The 5 GHz point for 1548 + 114 is from Pauliny-Toth and Kellerman⁷ and that for 1331 + 170 has been obtained at Westerbork by Ekers (private communication). The spectrum of 1442 + 101 is taken from Murdoch and Hoskins⁸ and further details are given there.

The source 1548 + 114 was first identified by Wills and Bolton⁹ with a 17^m BSO. There is a second 19^m BSO 5 arc s following in right ascension and also a diffuse red object of about 19^m which may be a galaxy about 10 arc s preceding the bright object. Both BSOs have been confirmed as QSOs¹. The optical positions measured with the Molonglo two co-ordinate measuring machine¹⁰ to an accuracy 0.5 arc s in each coordinate for the stellar objects and 1 arc s for the g are:

17 max QSO 15 h 48 min 21.20 s + $11^\circ 29' 47.0''$

19 max QSO 15 h 48 min 21.52 s + $11^\circ 29' 46.5''$

19 mag g? 15 h 48 min 20.53 s + $11^\circ 29' 45.1''$

The 408 MHz position obtained from various Molonglo measurements including a recent measurement by J. M. Sutton is 15 h 48 min 21.12 ± 0.15 s + $11^\circ 29' 53 \pm 3''$. This is marginally consistent with the bright QSO but does not favour either of the other objects. A one dimensional brightness distribution at Westerbork at 5.0 GHz (Ekers, private communication) shows that about two-thirds of the

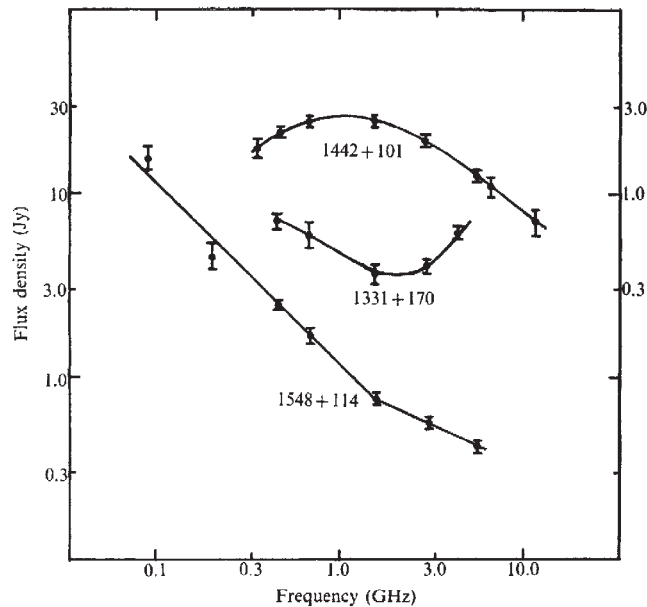


Fig. 1 Radio spectra of the three sources. The left flux density scale applies to 1548 + 114 and the right scale to 1442 + 101 and 1331 + 170. The flux density scale is in Jy. One Jansky = 10^{-26} W m $^{-2}$ s $^{-1}$.

flux density is contained in an unresolved (<3 arc s) component which coincides with the bright QSO within one arc second in right ascension. There is also an extended component (~ 20 arc s) about 10 arc s preceding in RA.

The spectral index from 80 MHz to 1,400 MHz is 1.00 ± 0.05 . From 1,400 MHz to 5 GHz the spectral index is 0.47 ± 0.09 . The two lines of fit are plotted in the figure but the change of slope must be more gradual than indicated. The best fitting single straight line over the range 80 to 5,000 MHz represents a spectral index of 0.80 but is a poor fit (χ^2 probability $\sim 5 \times 10^{-5}$). The spectrum is therefore of the centimetre excess (CE) type suggesting the existence of a compact component.

All of the observations used in plotting the spectrum were obtained with beams of some minutes of arc. The 408 MHz position suggests that most of the low frequency flux density comes from the bright QSO. Since the high resolution Westerbork data suggest the same at high frequency, the location of the CE component is not clear. It seems unlikely to be the extended component. The structure is probably more complex than the two components so far revealed.

The source 1331 + 170 has a strong centimetre excess suggesting a very compact component. The optical position is 13 h 31 min 10.00 s, $+17^\circ 04' 26''.0$.

The source 1442 + 101 is a QSO with a redshift of 3.53. Although the source peaks in the vicinity of 1 GHz there seems to be no evidence of centimetre excess emission up to 10 GHz. Of course, as a result of the high redshift, the peak emission in the rest frame of the source is close to 5 GHz.

By contrast, however, the secondary peak for 1331 + 170 ($z = 2.08$) must be greater than 15 GHz in the emission frame. Both 1331 + 170 and 1442 + 101 are reported to have rich absorption spectra, and both are highly luminous objects. Their radio spectra are consistent with the claim by Setti and Woltjer¹¹ that this region of the M - z plane is occupied only by sources with anomalous radio spectra.

The optical positions were measured on the two-coordinate measuring machine by Miss Sue Howard. I thank Dr R. D. Ekers and Dr E. J. Wampler for communicating their results prior to publication. The Molonglo Radio Observatory is supported by the Australian Research Grants Committee, the Sydney University Research Grants Committee and the

Science Foundation for Physics within the University of Sydney. The National Astronomy and Ionosphere Center and the National Radio Astronomy Center are thanked for observing facilities at Arecibo and Green Bank respectively. The National Radio Astronomy Observatory is operated by Associated University Inc. under contract to the National Science Foundation.

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Received December 10, 1973.

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Correlation search for dispersed radio emission from the galactic centre

WE report here the results of a search for fluctuations in the intensity of radio emission from the vicinity of the galactic centre using a two-channel cross-correlation technique.

The initial incentive to look for a time variable radio flux from the region of the galactic centre arose primarily¹ from the reports^{2,3} of impulsive gravitational radiation. Searches for impulsive radio events have been reported recently⁴⁻⁷. These observations have been restricted by the necessity to recognise individual pulses in the presence of fluctuation noise. They refer therefore to pulses substantially greater than the r.m.s. noise level. For example, Hughes and Retallack⁴ report pulses of height ≥ 85 f.u. at 858 MHz occurring at a rate of $\approx 10^{-4}$ s $^{-1}$. Other observers^{1,5,6} have utilized the expected delay in pulse arrival times at different frequencies due to velocity dispersion in the interstellar medium in order to reject impulsive noise of local origin.

We utilize the dispersion phenomenon in our search by looking for a delayed, correlated component to the intensity fluctuations at two neighbouring frequencies. Unlike those mentioned above, this method allows the detection of dispersed transients well below the r.m.s. fluctuation noise level. The technique is therefore particularly suited to the detection of low level signals, distinguishable only by their dispersed character. We describe its use in a search for dispersed noise having an arbitrary probability density function, for example pulses with amplitudes below those already reported⁴. The results of a concurrent examination of the data for individual large pulses will be reported elsewhere.

If $x(t)$ and $y(t)$ are the envelope detected outputs of the two telescope channels, their time lagged cross product is $R_{xy}(\tau) = \langle x(t) \cdot y(t + \tau) \rangle$, averaged over the observing time interval. The values of this mean lagged product are the cross-correlation estimates. Plotted as a function of the

time lag, τ , they constitute a correlogram which may be examined for evidence of a dispersed fluctuation noise component. Provided the characteristic time scale of the fluctuations is shorter than the time delay due to dispersion in the interstellar medium, a characteristic source signature, distinguishable from local noise, should appear in the correlogram. For non-periodic signals, a random pulse train for example, this signature takes the form of a positive peak in $R_{xy}(\tau)$ at a time lag, $\tau = \Delta t$, the time delay expected between channels separated in frequency by $\Delta\nu$ (MHz). $\Delta t(s) = 8 \times 10^3 DM (\Delta\nu/\nu^3)$ where DM is the dispersion measure of the variable source.

The height of the peak will be proportional to the mean square value of the correlated flux density variations. Its width indicates their time scale. The peak amplitude may be depressed and the characteristic width may be increased by: (1) dispersion within the non-zero channel bandwidths; (2) a non unique value of dispersion measure; (3) post-detector filtering; (4) decorrelation between the signals occurring at the source or in the medium.

The signal to r.m.s. noise ratio of a correlation estimate, $R_{xy}(\tau)$ increases as the square root of the product of the channel bandwidth and observing time. This indicates the high sensitivity of the correlation method for long observing times and wide bandwidths. In practice the presence of slow correlated instrumental drifts reduces the sensitivity below that expected.

The 150 m meridian transit telescope⁸ of the University of Otago, situated at the Invermay Radio Observatory near Dunedin, New Zealand (46°S) was used in the search. The telescope was operated at two frequencies, 120 MHz and 130 MHz as a two element phase switched interferometer in order to minimise interference and reduce instrumental drift. The effective aperture of the telescope is 5×10^2 m². The telescope was steered to declination $\delta = 29.0^\circ$ S for the two months search duration, from June through July 1973. The area of the search was a ($2^\circ \times 8^\circ$) strip centred on the galactic centre covering essentially the same region as that covered by Hughes and Retallack⁴. It included both Sgr A and the source they reported at $\alpha(1950) = 17^h 48.2$ min, $\delta(1950) = -28^\circ 58'$. The duration of transit between the half power points of the antenna beam was approximately 50 min. The system temperature reached a maximum of 2,200 K during the transit of the galactic centre. The r.m.s. temperature fluctuation was typically 1 K (6 f.u.) for a post detector integration time constant of 10 s. Calibration was performed periodically by noise injection at the antenna.

The receiver outputs, following envelope detection were low pass filtered with time constant 10 s, this value being approximately the pulse broadening time within the receiver bandwidth of 1 MHz expected for a hypothetical impulsive source having a dispersion measure of 2×10^3 cm⁻³pc. The integrated signals were then AC coupled with time constant of 100 s (in order to reduce slow drifts) to the data logging system. This consisted of a dual channel chart recorder used for visual detection of dispersed events and two dual channel magnetic tape recorders on which the signals were written in FM mode together with a stable clock reference signal used for synchronizing the tape read-out. The data were sampled at 0.30 Hz. Cross correlation analyses were performed on 30 min data blocks.

For the purposes of envelope correlation only those records of highest quality were used. Any 30 min blocks which contained non dispersed individual pulses greater than about 20 f.u. were rejected from this analysis. Twenty-four individual correlograms, representing 12 h (20% of all data) were computed for delay times, τ from -400 s to +400 s. A delay of $\tau = +400$ s corresponds to $DM = 10^4$ pc cm⁻³. Figure 1 shows the computed average of the twenty-four correlograms. The most noticeable feature is the positive peak

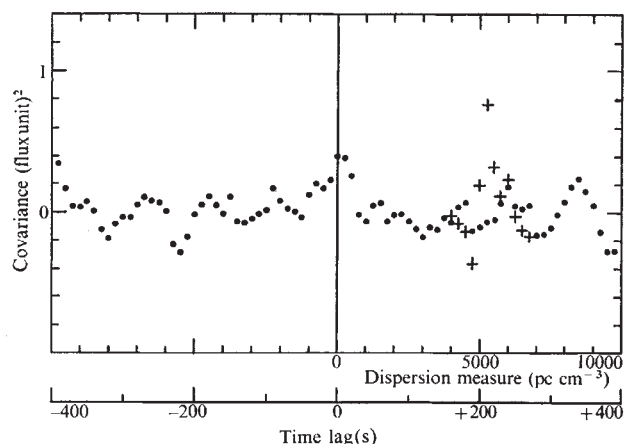


FIG. 1 Average cross-correlogram of galactic centre radio wave intensity fluctuations at frequencies of 120 and 130 MHz. Crosses indicate the signature due to a random sequence of dispersed ($DM = 5 \times 10^3$ pc cm⁻³) calibration pulses of variance 1.2(f.u.)².

at zero delay. This peak, 10^{-2} square degrees (4×10^{-1} f.u.)² in height indicates the presence, in spite of careful selection of data, of undispersed system temperature fluctuations with an r.m.s. value of 10^{-3} K. For time lags $|\tau| \gtrsim 20$ s ($DM \gtrsim 500$ pc cm⁻³) the cross covariance fluctuations have an r.m.s. value of 0.1(f.u.)². Any dispersed noise component with variance exceeding 2×10^{-2} (K)² corresponding to 0.8(f.u.)² for a source near the galactic centre, would be clearly visible.

To illustrate this and to check the computation procedures, a random sequence of dispersed pulses was generated, added to the raw data and processed. The resulting correlogram signature shown in Fig. 1 is consistent in location ($DM = 5 \times 10^3$), shape (full width at half maximum = 20 s) and covariance (0.8(f.u.)²) with that expected for these facsimile pulses. The mean pulse rate, $\alpha = 2.5 \times 10^{-2}$ s⁻¹, and the amplitude of each pulse was $s = 1.5$ f.u., approximately $\frac{1}{4}$ the r.m.s. noise level.

We conclude that a conservative upper limit of 0.8(f.u.)² may be assigned to the mean square flux density variations at 125 MHz of a variable source located either at the position of the impulsive source previously reported⁴ or in the direction of the galactic centre. This upper limit remains less than about 3(f.u.)² for sources with $\delta(1950) = 29.0 \pm 1.0^\circ$ S, and $\alpha(1950) = 17^h 44$ min ± 18 min. In order to attach a physical significance to our results and to relate them to other observations, we consider several sources of variability.

As a possible scenario we assume the previously reported pulses⁴ to be the chance superposition of more frequent, randomly occurring subpulses generated perhaps in stellar events in the region of the galactic centre. It is easy to calculate the variance $\sigma^2(s) = \langle s^2 \rangle - \langle s \rangle^2 = \alpha e^2/T$ and the mean flux density $\langle s \rangle = \alpha e$ for impulse, e , and resolving time, T . If the 'giant' pulses reported contain m unresolved subpulses in time T we may assume for illustrative purposes that, as reported $me = 8.5 \times 10^{-25}$ J m⁻² Hz⁻¹ (85 flux unit seconds), $T \sim 1$ s, and the 'm-fold' pulse rate $\alpha P(m-1, T) \approx 10^{-4}$ s⁻¹ where $P(m-1, T)$ is the Poisson probability of observing $(m-1)$ pulses in time interval T . For $m = 3$, $\alpha \approx 6 \times 10^{-2}$ s⁻¹, and $\sigma^2 \approx 50/T$ (f.u.)². A value of $T \approx 20$ s is appropriate to our observations at 125 MHz, taking into account post detector integration and convolution with the receiver bandwidths of dispersed impulsive generated by a source or sources having $DM \leq 5 \times 10^3$ pc cm⁻³. The amplitude of the correlogram signature would in this case be ≈ 2.5 (f.u.)², more than twice the amplitude of the facsimile signature shown in Fig. 1

and ≈ 25 times the r.m.s. correlogram noise. Other values of m do not significantly affect this result which clearly shows the 'giant' pulse model to be unlikely. Raising m , will in the limit, simulate a physical situation in which gaussian noise fluctuations are generated at the source. Our results applied, say to Sagittarius A, place a conservative upper limit to its correlated variance over a 10 MHz band centred at 125 MHz of $\sim 1(\text{f.u.})^2$ for periods from 50 to 500 s. This upper limit to the correlated variance of $1(\text{f.u.})^2$ may also be used to place a limit on the flare star contribution⁹ to the time averaged brightness temperature of the galactic centre region. If the flare stars are concentrated in a 2 (deg)² region of thickness Δ (DM) $\approx 10^3$ pc cm⁻³, a realistic distribution¹⁰ of flare event sizes then gives $\langle T_{\text{FS}} \rangle \leq 5$ K. This is a fractional contribution of $< 10^{-3}$ to the mean temperature of the region.

We acknowledge financial support from the New Zealand University Research Grant Committee.

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Received November 19, 1973.

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Near Infrared Observations of Cygnus X-3

THE system known as Cygnus X-3 has been extensively studied at radio wavelengths (where it exhibits extraordinary variability¹⁻³), in X rays (where it exhibits a 4.8 h period⁴⁻⁶), and in the infrared at 1.6 and 2.2 μm (where it also displays a 4.8 h period as well as large flares^{7,8}). Attempts have also been made to detect Cyg X-3 in visual and near infrared wavelengths^{9,10}. Here we present the results of a series of observations made at near infrared wavelengths, $\lambda_{\text{effective}} \approx 0.85 \mu\text{m}$ on ten nights in July and August 1973.

Our observations were made with a magnetically focused image tube, ITT F 4089, which has an S-25 photocathode that is sensitive in the near infrared. The image tube was placed behind a Wr-88A filter and the exposures were recorded on Kodak 103a D plates pressed against the output P-20 phosphor. Typical exposure times were 15 min at the 1-m reflector telescope of the Wise Observatory. This equipment is somewhat more sensitive in the near infrared than previous systems¹⁰ used for this problem and our coverage is much more extensive.

No object was detected to our plate limit at the position of the radio source Cygnus X-3. Our lower limits for the magnitudes at λ_{eff} vary from $I \sim 17.5$ mag to 18 mag depending on conditions; observations were made at the following universal times: July 3.95, 4.99, 6.97, 7.92, 8.83, 9.85 and August 1.03,

1.87, 3.98 and 6.90. The magnitude limits given above are crude estimates based upon plates, taken with the same equipment, of M15 (Sandage¹¹ has reported accurate *UBV* photometry for M15) and the relationship between *B*, *V* and *I* colours given by Johnson¹². It should be noted that the 4.8 h periodicity of Cyg X-3 was studied on July 9.2–9.55 by Becklin *et al.* (ref 8) at 1.6 μm and 2.2 μm (with the Hale 200-inch telescope) and at X-ray wavelengths (with the Copernicus satellite). Their observations took place between two of our exposures listed above. It should also be noted that a radio event apparently occurred in Cyg X-3 near the beginning of our observation period in July¹³ and our results therefore limit the near infrared strength of this event.

Our upper limits on the near infrared radiation from Cyg X-3 can be compared with expectations based on simple models. The expected *I* magnitude ($\lambda_{\text{eff}} = 0.9 \mu\text{m}$) is

$$m_I = m_K + E_{I-K} + (M_I - M_K)$$

where⁷ $m_K = 11.4$ mag, E_{I-K} is the selective absorption, and ($M_I - M_K$) is the difference in absolute magnitudes between $\lambda = 0.9 \mu\text{m}$ and $2.2 \mu\text{m}$. The selective absorption can be estimated to be $E_{I-K} \approx 6$ mag by using the obvious relation $E_{I-K} = (E_{H-K})(E_{I-K}/E_{H-K})$, the observational determination that⁷ $E_{H-K} \approx 1.22 \pm 0.1$ mag and the average (≈ 4.9) of four separate determinations of E_{I-K}/E_{H-K} (4.25 in the direction of Aquilla-Cygnus¹⁴, 5.43 to the Galactic centre¹⁵ and 4.48 and 5.30 from measurements involving M supergiants¹⁶). For a typical early-type supergiant such as has been associated with a number of other X-ray sources, one has $M_I - M_K = -0.5$ mag. Thus a typical value to have expected for m_I would have been $m_I \sim 16.9$ mag (with more than 0.5 mag uncertainty due to the imprecisely estimated selective absorption). Our results are consistent with, but do not completely establish because of uncertainties in selective absorption and plate limits, a spectrum that is flatter than the $f_\nu \sim \nu^2$ associated with a supergiant in this wavelength region. It will be important to attempt to obtain in the next observing season a better determination of the selective absorption to Cyg X-3 in order to establish firm limits on the shape of the spectrum.

The expected visual magnitude for Cyg X-3 is $m_V = 24.8$ mag if one uses results in the direction of Aquilla-Cygnus¹⁴ or the Galactic centre¹⁵ to calculate the selective absorption and $m_V = 28.5$ mag if one uses the average result obtained with M-supergiants¹⁶. In both cases, we have assumed $M_V - M_K = -0.9$ mag corresponding to an early O or B star¹⁴. If we had assumed a flatter spectrum, we would of course have obtained fainter values of m_V , for example, ~ 1 mag fainter for an AO star. The estimated values for m_V are all consistent with the limit of $m_V = 23.9$ mag set by Westphal *et al.*⁹. The estimated total visual absorption varies between $A_V = 18.3$ mag to 20.4 mag, depending on how one calculates the selective absorption.

We thank Y. Avni, E. Becklin, J. Katz, and G. Neugebauer for valuable discussions and P. Veron and P. Wehinger for developing the image-tube system for direct photography. We acknowledge the Wise Observatory of Tel-Aviv University for the use of their facilities at Mitzpeh Ramon, Israel, and a Smithsonian research grant. This research was supported by the National Science Foundation.

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Extended Glow Preceding Reappearance of Saturn during a Lunar Occultation

THE recent occultation of Saturn by the Moon¹, visible across Canada in the early morning of Wednesday, October 17, 1973 provided the opportunity to observe and photograph the reappearance of the planet from behind the dark lunar limb. Teams of observers from the Calgary Centre of the Royal Astronomical Society of Canada and the University of Calgary Physics Department established telescopes at various sites in Alberta but poor weather restricted photography of this event to times rather late in the reappearance and to a "close approach" sequence at the University of Calgary Rothney Astrophysical Observatory south of the shadow path.

The present observation was made from the northernmost site at Ardrossan, near Edmonton, Alberta (53° 33' 45" N, 113° 07' 50" W) where a team of four observers were spaced along a line at right angles to the shadow path several hundred metres apart in an effort to observe photoelectrically as well as visually the grazing occultation of the 8 mag Titan by the south lunar limb. This attempt was thwarted by instrument problems and by intermittent clouds, but the sky cleared considerably by the predicted time of Saturn reappearance with only light haze surrounding the 66% sunlit Moon. Cameras were mounted on three of the four telescopes at this site and observers were linked by intercom to each other and to a tape recorder on which WWV time signals as well as observer comments were recorded. The fourth observer was to provide careful visual observations and detailed timing of the reappearance and was equipped with a 6 inch *f*/9 Newtonian telescope equipped with a 12 mm eyepiece and a $\times 2$ Barlow lens (overall magnification $\times 230$) to ensure that light from the bright limb did not obscure the observation in the 12 arc min field of view at the moment of egress.

The observation upon which this report is based was made by the visual observer at a time between 1 s and 2 s before the appearance at the dark lunar limb of the first pin-point of light

from Saturn's rings. At this time, the observer's eye was attracted to a faint glow which he probably first saw by averted vision but which delineated the dark limb and which appeared like seeing "a campfire on the other side of a treeless hill on a dark night". The extent of the glow was estimated afterwards to have been a little greater than the east-west extent of the rings. The pin-point of light from the rings which appeared in the centre of the faint glow rapidly brightened and became bigger as the rings became visible following the third contact. The glow increased slightly in width and showed easily detectable Moon limb curvature on its sharp edge but at about 4 s beyond third contact the glow was indistinguishable against the increasing ring brilliance.

Superficial explanations for this glow can be quickly dismissed. If internal reflection within the telescope of light from the bright lunar surface had caused the glow, it was certainly a coincidence that it not only appeared in the exact position where the rings showed themselves but also was aligned with the direction and position of the dark Moon edge. Mist in the Earth's atmosphere could have produced a hazy glow just as the first light of the rings appeared but this would almost certainly have been circular from the initially observed edge of the rings. This must remain a distinct possibility but it is at least as tempting to associate this glow with the planet and a tenuous outer atmosphere surrounding Saturn and its rings which is normally invisible against the planet's brightness. Observation of a faint outer ring (the so-called D ring) has been referred to several times this century (see for example, Guerin²) but not confirmed by modern photography. These observations may be related to the present result. Furthermore a very similar observation to that seen for Saturn was reported by Brock³ for the planet Jupiter when it was occulted by the south-limb mountains of the Moon. At that time a glow was noted to be moving along above the mountain range during the grazing occultation. This glow associated with Jupiter was reported to vanish immediately the planet appeared and it was not seen by observers situated one quarter of a mile north and south of the observer.

Thus, a tentative conclusion for these unique visual observations must be that these giant planets have extended atmospheres associated with them, an idea suggested for Jupiter by Peek⁴ who postulated an upper atmosphere consisting of light elements and extending to great heights above the cloud layers. Estimates of total pressure of gas above the cloudtops from spectroscopic data vary from 2 to about 13 atm (ref. 5). Such gaseous atmospheres would not be expected to scatter sunlight but could presumably emit light equivalent to airglow or even auroral luminosity under the action of the solar wind. The existence of a magnetosphere and Van Allen belts is inferred from the intense decimetric radio bursts detected from Jupiter⁶. The requisite particle flux for excitation of such luminosity is thus present, at least for Jupiter. Radio bursts from Saturn are much weaker but the similarity of this planet to Jupiter would lead one to believe that such luminosity is possible from Saturn also.

In the case of the present observation of Saturn, if we assume 1.5 s for the time between appearance of the glow and that of the rings, the extension beyond the rings is approximately 5,000 km or 4% of the planet's diameter. The observer's reports of the glow accompanying Jupiter would indicate a glow extending about 1,000 km above the cloud tops of Jupiter, or less than 1% of the planet's diameter. The geometry of these events was such that the glow was observed close to the polar regions of both planets.

It is obvious that repeated measurements of this phenomenon, particularly careful photoelectric observations, are necessary in order to verify the existence of the glows but these can be made only very infrequently. Of much greater importance, bearing in mind the approach of the Pioneer spacecraft to Jupiter, is that extended glows should be searched for in data from these deep space probes.

The authors acknowledge the assistance given to them by U. Haasdyk and C. Treleaven during the present observations.

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Interpretation of Solar Hard X-ray Burst Polarisation Measurements

TINDO *et al.* (refs 1 to 3 and unpublished results) have reported the first observations of polarisation of solar flare emission in the hard X-ray band (around 15 keV) and have made a preliminary interpretation of these³. Their results depend, however, on the removal of systematic differences in sensitivity of the detectors used in the Intercomos Thomson-scattering polarimeters, by calibration on an 'unpolarised' source. We point out here that the results as presented by Tindo *et al.* are in fact in error since their calibration was based on the assumption that the hard X-ray flux from the flare itself tends to zero polarisation in its final stages of decay. This assumption is invalidated by the presence, in the total flux, of a large polarised contribution from photospheric albedo photons^{4,5}. We further consider how much this calibration error may affect interpretation of the Intercomos results in terms of flare particles and suggest how further theoretical work, combined with results from a laboratory calibrated polarimeter, can yield information on the spatial location of hard X-ray flares as well as on their true polarisation.

Hard X-ray polarisation measurements have a key role to play in the formulation of flare theories since the degree and plane of polarisation indicate the velocity distribution of the bremsstrahlung source electrons—in particular, whether they stream along the flare field lines or are magnetically trapped—thus giving insight into the flare particle acceleration mechanism^{3,6,7}. Yet more important, future observations of the variation of the polarisation with photon energy will provide a means of determining the photon energy above which non-thermal electrons rather than the multi-temperature flare plasma is the dominant X-ray production mechanism⁸. This question is fundamental to the overall energetics of a flare due to the characteristically steep electron spectra involved. Thus if the thermal component dominates to about 30 keV, the electron streams responsible for the emission above this energy would carry only a small proportion of the total flare energy and so be a minor artefact of the release process. On the other hand, if non-thermal emission dominates to below 10 keV, these streams carry quite adequate energy to supply the entire thermal flare and so may be fundamental to flare heating⁹.

In the two main models of hard X-ray flares, the primary bremsstrahlung flux is partially linearly polarised either along or across the solar disk radius through the source^{3,6,7} and so may be expressed in terms of its components $F_{1\parallel}$ and $F_{1\perp}$ in

these respective directions. Tomblin⁴ and Santangelo *et al.*⁵ have, however, emphasised the importance of photons back-scattered from the photosphere which, in the deka-keV range, may be reflected with efficiencies up to 80%. Santangelo *et al.*⁵ have further pointed out that this albedo contribution will be polarised by the scattering, so modifying the polarisation of the total X-ray flux observed and even introducing polarisation⁸ into the flux originating in unpolarised primary emission such as a multi-thermal source⁸ or a non-thermal one in which the velocities are randomised. The degree of this scattering polarisation has so far only been calculated¹⁰ in the case of low photon energies where it is $\lesssim 2\%$. At these energies, however, the albedo contribution to the total flux is itself small due to photo-electric absorption^{4,5}. In the deka-keV range the process is much nearer to pure scattering for which the polarisation in the scattered component itself may approach 100% for 90° scattering, a figure not much reduced even for multiple scattering¹² as involved here. (This situation is very similar to the optical polarisation of the blue sky^{11,12}.) The plane of maximum intensity in this scattering polarisation will always lie transverse to the solar radius through the source^{5,8}. Thus if the photospheric albedos are $\rho_{\parallel}$ and $\rho_{\perp}$ respectively for radiation polarised in the radial and transverse directions (depending on the photon energy and the scattering geometry), the secondary (reflected) contributions to the observed total fluxes are

$$F_{2\parallel} = D_{\parallel} \rho_{\parallel} F_{1\parallel} \quad (1)$$

and

$$F_{2\perp} = D_{\perp} \rho_{\perp} F_{1\perp}$$

Here $D_{\parallel}$ and $D_{\perp}$ measure the directivity of the primary source (for the respective polarisation planes) in the direction of the photospheric scattering centre as compared to the Earth direction. (Inclusion of these two parameters is essential since any non-zero primary source polarisation will in general be accompanied by a source directivity and a variation of the polarisation with direction^{6,7}—that is, $D_{\parallel} \neq D_{\perp} \neq 1$ except in special cases.) Total observed fluxes in the two planes are therefore

$$F_{T\parallel} = F_{1\parallel}(1 + \rho_{\parallel} D_{\parallel}) \quad (2)$$

and

$$F_{T\perp} = F_{1\perp}(1 + \rho_{\perp} D_{\perp})$$

where $\rho_{\parallel}$ and $\rho_{\perp}$ may be taken as constant through the burst while $D_{\parallel}$ and $D_{\perp}$, as well as $F_{1\parallel}$ and $F_{1\perp}$, will vary during its development.

The physical basis of the calibration method of Tindo *et al.* (refs 1 to 3 and unpublished results) is that, late in the burst, source electron velocities become randomised so that the intrinsic polarisation tends to zero ($F_{1\parallel} = F_{1\perp}$). (This also implies $D_{\perp} = D_{\parallel} = 1$.) This is reasonable but, in applying it, Tindo *et al.* have neglected the albedo contributions in equation (2) which are present during calibration (as at all times); that is, they have taken $F_{T\parallel} = F_{T\perp}$ and attributed the imbalance in their detector count rates entirely to differing sensitivities. Thus, if we suppose for the present purpose that the calibration was done in terms of correction of the radial ($\parallel$) count rate relative to the transverse ($\perp$), then all the transverse count rates from the experiment have been reduced by a calibration factor

$$1/\beta^* = 1/\beta\gamma \quad \text{where } \gamma = (1 + \rho_{\perp})/(1 + \rho_{\parallel}) \quad (3)$$

instead of by $1/\beta$ where β is the true transverse/radial counter sensitivity ratio. Since, for 90° scattering, $\rho_{\perp}$ may tend to 1 and $\rho_{\parallel}$ to 0, γ may be as high as 2, implying a serious discrepancy between the polarisations deduced by Tindo *et al.*³ and those actually present in the total flux from the flare. In the late decay phase, for example, the polarisation in the observed flux due to scattering would amount to

$$p_s = (F_{T\perp} - F_{T\parallel})/(F_{T\perp} + F_{T\parallel}) = (\rho_{\perp} - \rho_{\parallel})/(2 + \rho_{\perp} + \rho_{\parallel})$$

which could be as high as 1/3 (for 90° scattering) rather than the zero value attributed in the experiment calibration.

The basic aim of the Intercosmos experiments was to determine the polarisation characteristics of the primary X-ray burst source. Correction of the results of Tindo *et al.*³ for albedo effects will require further theoretical work on the scattering process, but some tentative conclusions may be drawn regarding the extent to which their results deviate from this intrinsic polarisation. This latter may be written as $P_1 = (r_1 - 1)/(r_1 + 1)$, where $r_1 = F_{1\perp}/F_{1\parallel}$, whereas the false polarisation deduced by Tindo *et al.* is $P_0 = (r_0 - 1)/(r_0 + 1)$ where r_0 is related to r_1 by equations (2) and (3), namely

$$r_0 = r_1(1 + \rho_{\parallel} D_{\parallel}) / (1 + \rho_{\parallel}) \times (1 + \rho_{\perp}) / (1 + \rho_{\perp} D_{\perp}) \quad (4)$$

First, it is interesting to note that if source directivity were neglected in equation (4), then $D_{\parallel} = D_{\perp} = 1$ and $r_0 = r_1$ so that the results presented by Tindo *et al.*³ would indeed represent the primary source polarisation, though not the polarisation actually present in the total flux observed. This coincidence arises because the fractional albedo contribution would remain the same, in this case, between calibration and the burst itself. In practice, however, neither the polarisation nor the intensity of the primary emission will be the same in the Earth and photospheric directions, except by special coincidence. As a realistic example of primary emission characteristics we may consider the thick target model (analysed by Brown⁷) in which an electron stream bombards the low chromosphere. For a flare at CMD=45° (90° scattering), this model predicts $r_1 \approx 0.6$ and $D \approx 4.8$ for 15 keV photons, giving $r_0 = 0.21$ by equation (4) (with $\rho_{\perp} = 1$, $\rho_{\parallel} = 0$). The intrinsic source polarisation is thus $P_1 = -0.25$ as against the value $P_0 = -0.5$ which would have been deduced by Tindo *et al.* in this idealised case. For different scattering geometries and photon energies it is quite feasible that neglect of the albedo contribution might result in deduction of the incorrect plane of maximum intensity as well as degree of polarisation.

In conclusion it is clear that no definitive interpretation of hard X-ray flare polarisation measurements may be made without full theoretical investigation of $\rho_{\parallel}$ and $\rho_{\perp}$ which depend on the scattering geometry, and so on the unknown location of the primary source in the solar atmosphere. If, however, future experiments are conducted using laboratory-calibrated detectors then the polarisation observed late in the flare can be attributed to photospheric scattering and, by comparison with theory, could be a valuable tool in inferring the primary source location without direct angular resolution. Once this is known, the albedo contribution may be subtracted from observations during the burst itself and the polarisation of the primary source deduced.

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Meteor of August 10, 1972

AN extraordinarily large and brilliant meteor was seen by many observers¹ in the western United States and Canada at 2030 UT (1430 local time), on August 10, 1972. A satellite-borne near infrared radiometer also detected and tracked it. W. T. Rogers, a surveyor of Billings, Montana, obtained a ground based sighting which provided additional altitude information. Using his measurement and the satellite data we calculated the trajectory.

The meteor first became sufficiently hot to be detected over Utah and it finally cooled below the detection threshold over Alberta, 100 s and 1,500 km later². Its path is shown in Fig. 1. The meteor very narrowly missed hitting the Earth, its perigee being only 58 km. It apparently did not fragment. Quantitative trajectory parameters are given in the table. Because of the availability of the satellite data the accuracy of this trajectory far exceeds that determined for any other meteor.

Table 1 Meteor Trajectory

Observation	GMT (s)	Velocity (km s ⁻¹)	Geodetic altitude (km)	Geodetic latitude north	Geodetic longitude west
Initial	73708.9	15.03	75.98	38.389	112.575
Perigee	73747.5	14.60	57.85	44.386	112.993
Final	73810.1	14.21	101.55	53.331	113.785

We obtained estimates of the mass, kinetic energy and size, using the velocities from Table 1 and the measured infrared radiation. We equated the loss in kinetic energy to the thermal radiation. This neglects the small fraction of the energy converted into acoustic waves. We calculated the integrated

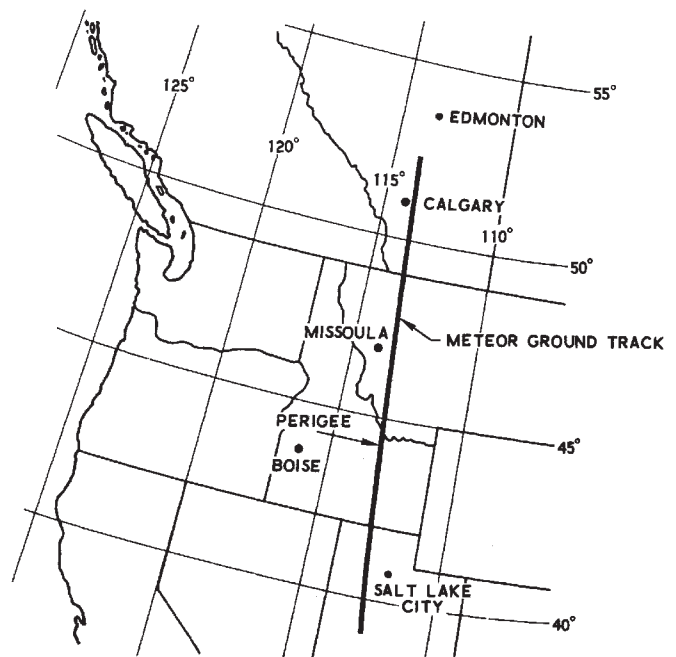


Fig. 1 Path of meteor of August 10, 1972.

thermal radiation from that within the infrared passband assuming that the source could be approximated by a 3,000 K blackbody. We believe that the resultant thermal energy is accurate to within a factor of four, with most of the error arising from the temperature and blackbody assumptions. The mass turned out to be 10^9 g or about 2% of the estimated mass of the Barringer meteor which made the Arizona Crater. The calculated equivalent diameter is 4 m, assuming the density of iron. The initial kinetic energy was 10^{14} J, or approximately the yield of the nuclear weapons which destroyed Hiroshima and Nagasaki. The meteor approached the Earth at 15 km s^{-1} and if it had been at a slightly lower altitude, the damage would have been very extensive. Fortunately meteors of this size are exceedingly rare.

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Aftershocks Caused by the Novaya Zemlya Explosion on October 27, 1973

In the autumn of 1973 seismic signals were recorded from three underground nuclear explosions on Novaya Zemlya in the Arctic Ocean. Two of these explosions were located at a new site on the southwestern part of Novaya Zemlya. The explosion on October 27 at the new site was followed by a suit of 10 small events, all of which occurred within 4 h of the explosion. Here we give the locations of the explosions and the afterevents based on data from Nordic and North American stations and the discrimination results based on shortperiod data obtained at the Hagfors Observatory¹ (HFS) in Sweden.

Table 1 summarises pertinent location data with their error estimates for the ten afterevents, the 1973 explosions and an explosion in 1969 used in the discrimination study. The epicentres are obtained by a joint epicentre location technique² utilising arrival times of seismic P and S phases. The location errors given as standard deviations include error estimates of the traveltimes model and should thus be regarded as fairly realistic estimates of the actual errors. Arrival time data reported by Nordic and North American stations are used. Epicentre data for two explosions reported by NOAA³ are also given in Table 1. The explosions on September 27 and October 27 are both located about 200 km south of the main test site of Novaya Zemlya, where the explosion on September 12 was fired. The map (Fig. 1) shows the locations of the main test site close to the channel between the northern and the southern island and the new test site in the southwestern part of the island.

Figure 2 displays the shortperiod P-wave signals recorded at HFS from the explosions in 1973 and seven of the afterevents. No surface waves for the afterevents are detected at HFS. The body wave magnitudes, m_b (HFS), which are supplemented in Table 1, range over four orders of magnitudes. Four magnitudes correspond to a dynamic range of 80 dB which puts rather strong requirements on the recording equipment. To illustrate the dynamic range of seismic signals, the signals from the three explosions, cover-

ing a range of two magnitudes or 40 dB, are plotted in the same scale. The afterevents are plotted with a gain of roughly 500 times that of the explosions. Figure 2 shows the simple, pulslike waveforms of the explosions⁴ compared with the more complex afterevent signals.

To study the differences of the P-signal spectral content between the explosions and the afterevents the third moment of frequency (TMF) parameter, which is a measure of the high frequency content of the signals, is calculated for the signals recorded at HFS. TMF has previously been used to discriminate between explosions and earthquakes^{5,6}. The TMF values are given in Table 1. The high frequency content is, however, not only a function of the source type but also of the magnitude of the event. This magnitude effect is clearly demonstrated in Fig. 3 where TMF is plotted against m_b (HFS). The trend for the Novaya Zemlya explosions covering two orders of magnitudes is very clear. Extrapolating this trend down to magnitudes comparable with those of the afterevents would yield a very large difference between the two types of events. This difference indicates that the explosions and the afterevents are different types of seismic sources.

As far as we know no seismic activity has been reported in the Novaya Zemlya region nor has any of the earlier explosions been followed by aftershocks. Aftershock activities have however been reported following high yield explosions on the Aleutian Islands⁷ and at the Nevada Test Site^{8,9}. The events observed in these regions are of two different kinds. One type is associated with the deterioration of the cavity and these events are located in the immediate vicinity of the cavity. Such events have been observed soon after the explosion and they continue until the cavity completely collapses. The second type of events is the explosion stimulated tectonic events. These may occur at distances greater than 10 km from the explosions and such events have been observed months after the explosions. The tectonic events seem generally to be weaker than those related to the cavity deterioration. The numbers of after-

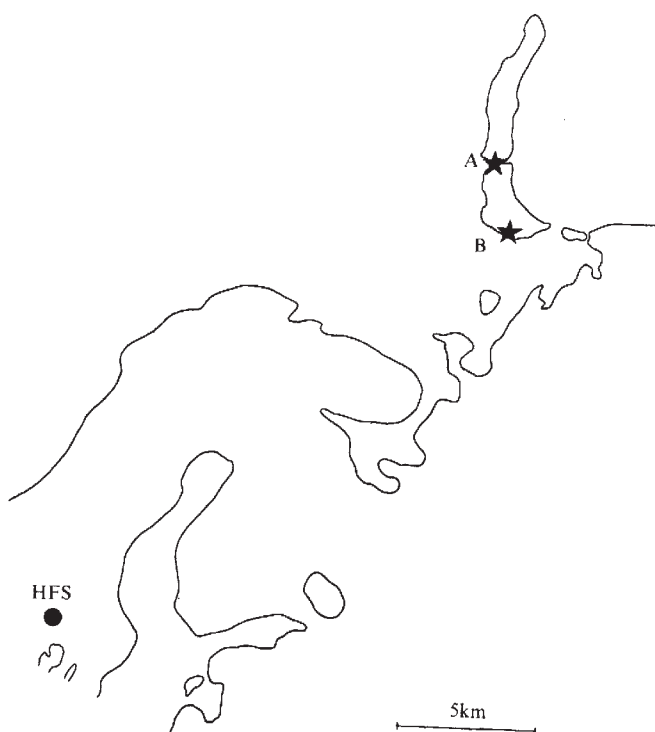


Fig. 1 Relative locations of the main (A) and the new (B) test sites on Novaya Zemlya and Hagfors Observatory (HFS).

Table 1 Location data of Novaya Zemlya events

	Year	Date Month	Day	Origin time			Lat (N)	Long (E)	No. of stations in epicentre location	NOAA		m_b (HFS)	TMF (HFS)
				h	min	s				Lat (N)	Long (E)		
Explosion	69	10	14	07	00	07	73.4 ± 0.1	54.7 ± 0.3	14	73.4	54.8	6.7	11.0
	73	09	12	06	59	54	73.1 ± 0.1	55.6 ± 0.3	13			7.8	6.0
	73	09	27	06	59	58	70.7 ± 0.1	53.9 ± 0.3	11	70.8	53.9	6.0	14.9
	73	10	27	06	59	57	70.8 ± 0.1	54.1 ± 0.3	12			7.3	8.1
Afterevent	73	10	27	07	52	25	71.1 ± 0.2	54.0 ± 0.6	6			4.0	10.5
				08	03	58	71.0 ± 0.2	54.0 ± 0.6	8			4.1	12.3
				08	09	35	70.7 ± 0.3	54.8 ± 0.9	6			3.8	13.6
				08	21	21	71.0 ± 0.2	54.0 ± 0.5	10			4.4	11.0
				08	23	59	70.5 ± 1.2	55.2 ± 2.0	5				†
				08	31	38*	70.8 ± 0.5	55.4 ± 2.0	4				†
				08	56	01*	71.4 ± 0.3	53.2 ± 0.8	7			3.9	9.7
				09	01	29	70.2 ± 0.5	53.4 ± 2.5	3				†
				09	13	49	71.1 ± 0.2	53.8 ± 0.4	11			4.4	10.1
				10	30	30*	71.8 ± 0.5	57.8 ± 1.9	5			3.7	13.2

* Some stations have larger arrival time residuals than usual which means that the actual error might be larger than that given in the table.
† Signals too weak to be analysed.

shocks detected by local seismic station networks from the deterioration of explosion cavities are very large. The Milrow and Cannikin explosions in the Aleutian Islands were followed by hundreds of events within a few hours of the explosions. These events were all small, most of them had seismic amplitudes less than 1/1,000 of those of the explosion⁷. These events are relatively weak compared to the events observed after high yield explosions at the Nevada Test Site, where the explosion Jorum for example generated fifteen events with amplitudes larger than 1/100 and forty events with amplitudes larger than 1/1,000 of the amplitude of the explosion⁹. The signals recorded by local networks from events related to the deterioration of the

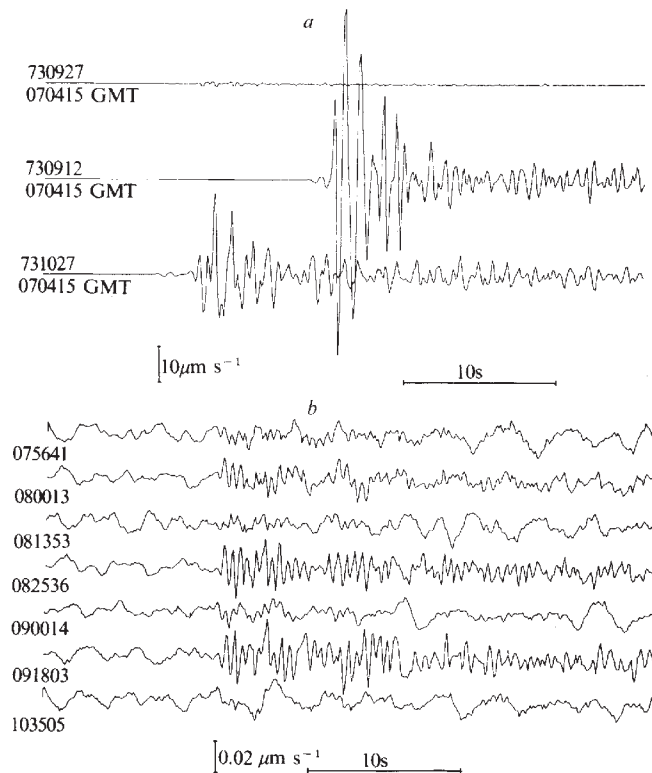


Fig. 2 *a*, Short period P signals recorded at the Hagfors Observatory in Sweden from Novaya Zemlya explosions; *b*, short period P signals recorded at the Hagfors Observatory in Sweden from aftershocks caused by the Novaya Zemlya explosion on October 27, 1973.

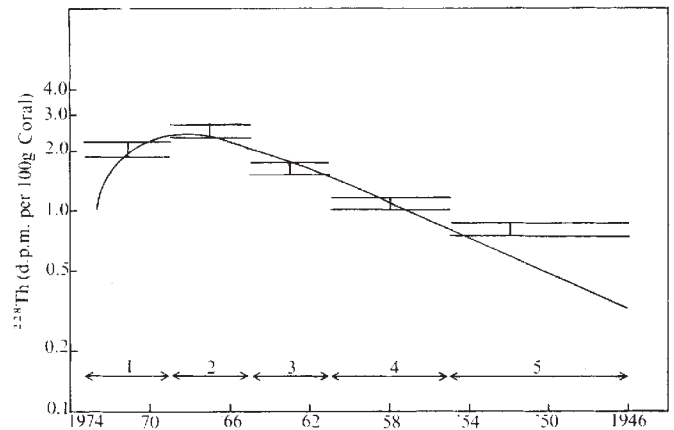


Fig. 3 Third moment of frequency, TMF, values as a function of body wave magnitude, m_b (HFS), for explosions (x) and aftershocks (o).

cavities were found to have a characteristic low frequency signature whereas tectonic events contain higher frequencies⁷. Significant spectral differences between teleseismic P waves from explosions and aftershocks associated with the explosion cavities at NTS have been reported¹⁰.

The aftershocks reported here from the Novaya Zemlya explosion on October 27 have the same general characteristics, maximum signal amplitudes of about 1/1,000 of that of the explosion and low frequency signals, as the aftershocks associated with the cavity deteriorations of the explosions in the Aleutian Islands. We thus conclude that the observed signals come from events related to the deterioration of the cavity of the explosion on October 27. No event which can be associated with the complete collapse of the cavity has so far been observed. The fact that no aftershocks have been observed from earlier large explosions at the old Novaya Zemlya test site might be explained by the difference in geology between the old and the new test site¹¹.

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Microfossils from the Late Precambrian Altyn Formation of Montana

SEDIMENTARY rocks of the late Precambrian Belt Supergroup are exposed in western Montana, Idaho, and adjacent parts of Canada. There are few reports of microfossils from the Belt Supergroup, although Metazoan ancestors may occur in the younger, upper Belt Missoula Group¹. Early this century Walcott² concluded that stromatolites found in the Belt were produced by blue-green algae. He reported pyritised blue-green algal filaments, associated with stromatolites, from the Newland Limestone of lower Belt age. Recent re-examination of this material supported Walcott's interpretation³. I report here the discovery of microfossils, which are older than 1,300 m.y., from the lower Belt Altyn Formation.

The Altyn Formation is exposed only in the eastern part of Glacier National Park, Montana, and adjacent areas of Waterton Lakes Park, Canada. The formation is thrust over Cretaceous rocks along the Lewis Thrust, and the original sedimentary base is unknown. Elsewhere in Montana the Belt Supergroup rests unconformably on igneous and metamorphic basement rocks⁴. The Altyn Formation belongs to the lower Belt or Pre-Ravalli and is overlain by the progressively younger stratigraphic sequence: Ravalli Group, Helena Dolomite, Missoula Group. The formation is mainly dolomitic, with abundant terrigenous detritus at some horizons. It contains desiccation cracks, intraformational conglomerates, algal laminated sediments, ooids and algal stromatolites; indicating deposition in a shallow, nearshore marine environment. The Altyn Formation is a shoreline facies, deposited at the eastern margin of the Belt sea, and it grades westward into a finer-grained siliceous clastic facies⁵. Stromatolites, the product of a combination of physical and algal processes, have been known from the Altyn Formation for many years⁶, but no fossil remains have been reported to date.

The Belt sediments were deposited during a 600 m.y. time span, between about 1,450 and 850 m.y. ago⁴, with the Altyn Formation belonging to the lower, older part of the sequence. The Altyn Formation is not dated radiometrically, but some indication of its age may be obtained by considering radiometric ages of associated or correlative formations. The overlying Missoula Group is at least 1,100 m.y. old⁷⁻⁹, and the Altyn Formation is some 2,700 m lower in the stratigraphic sequence¹⁰, and must be older. The Aldrich Formation of British Columbia, a stratigraphic equivalent of the Altyn Formation, is intruded by the Hell-roaring Greek granite which is at least 1,300 m.y. old¹¹. A second correlative of the Altyn Formation, the Prichard Formation in the Alberton,

Montana, area, underwent regional metamorphism 1,330 ± 45 m.y. ago^{4,9}. A suite of rocks from the Lower Belt of the Little Belt and Big Belt mountains has an age of 1,325 ± 15 m.y.⁹. Thus the Altyn Formation is at least 1,300 m.y. old, but is younger than the 1,700 m.y. old basement gneisses exposed beneath the Belt Supergroup in some places⁴.

The microfossils occur in a siliceous grain (Fig. 1a), in a clastic dolarenite. The fossils were discovered in a thin section of rock, approximately 30 µm thick, prepared for petrographic, microscopic analysis. This implies that the fossils are an inherent part of the rock and not a later contaminant. Further evidence for this is the partial replacement of some filaments by early diagenetic dolomite (Fig. 1a, b), and the truncation of part of the thallus at the grain margin (Fig. 1a). The microfossils thus antedate the formation of the grain and the diagenesis of the sediments. The rock was sampled from a sequence of interbedded clastic dolarenites and algal laminated, finegrained dolomiticrites, exposed near the top of Appekunny Falls, Glacier National Park, Montana.

The microfossils were preserved by the primary precipitation of amorphous silica. Subsequent recrystallisation to quartz provided a chemically and physically resistant medium for the organic material.¹² The microfossils demonstrate the effectiveness of this mode of preservation. The circular transverse sections show no evidence of distortion or compaction (Fig. 1c). The fossiliferous grain has withstood erosion and some intrabasinal transportation before its final deposition and lithification.

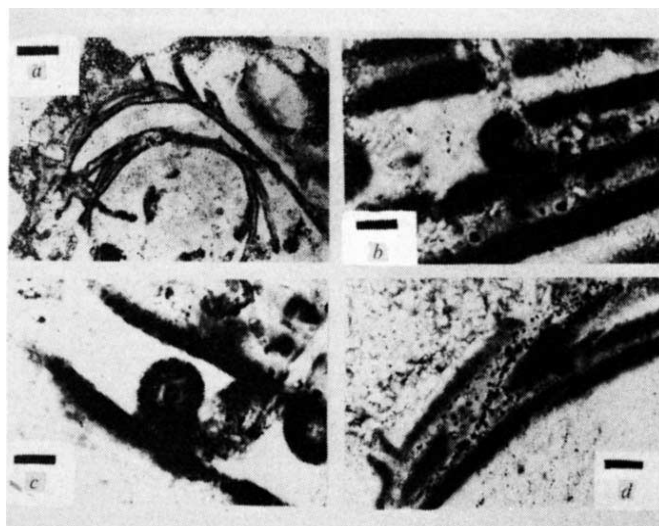


Fig. 1 Photomicrographs showing morphology of microfossils from the Altyn Formation. Bar scale: a, 100 µm; b and c, 10 µm; d, 20 µm.

The microfossils occur as a loosely coiled thallus composed of yellow-brown filaments. The filaments are tubular, non-septate, branched, occasionally straight, but more commonly curved (Fig. 1a), usually with rounded ends (Fig. 1b). Filaments are up to 1 mm long (incomplete filament) and circular in transverse section, with an average diameter of 16 µm (Fig. 1c). The filament walls have a quite uniform thickness averaging 6 microns. The filaments branch occasionally, with the angle between the two branches ranging from 15 to 90° (Fig. 1d). Intrafilament cellular material is not preserved, and mode of reproduction and the precise nature of the branching are unknown.

Comparison with previously described Precambrian microfossils reveals no forms which are directly comparable to the Altyn Formation microfossils. They are larger than *Archaeo- restis schreiberensis*, a tubular, branched, non-septate filament of unknown affinities found in the Gunflint chert¹³. Fossils of the genus *Eomycetopsis* from the Bitter Springs Forma-

tion of Australia, and of unknown but possibly fungal affinities¹², are similar in some respects to the Altyn material. They are tubular, cylindrical, predominantly nonseptate filaments; however, they are unbranched and have filament diameters less than one fourth of those found in the Altyn Formation microfossils. Tubular, nonseptate fossils of the genus *Siphonophycus* from the Bitter Springs Formation are believed to be sheaths of a blue-green alga¹³. The Altyn Formation microfossils differ from those belonging to the *Siphonophycus* in their somewhat larger size and in being branched. In the absence of preserved intrafilament cells it is not possible to determine the type of branching. True branching is typical of eukaryotic plants, but did not develop in blue-green algae until the Devonian¹⁴. Definite assignment of the Altyn Formation microfossils is not yet possible, but it seems likely that they may be the preserved sheaths of blue-green algae with false branching.

I thank G. W. deVillafraanca for use of his equipment and D. A. Haskell for information about Cyanophyta. Field and laboratory work supported by a Geological Society of America Penrose grant and by grants from the Aid to Faculty Scholarship Committee of Smith College.

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Received November 13, 1973.

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Calabrian Ridge, a newly discovered branch of the Mediterranean Ridge

Long range side-scan sonar surveys made from RRS Discovery during 1971 and 1973 have provided the first regional view of the structural grain of the Mediterranean Ridge, whose distinctive surface relief was known previously only from echo-soundings¹⁻³. The sonar surveys clearly show that there is an important grain extending along much of the Ridge, but also there is considerable local variation and other trends are well developed in places. Individual ridges and troughs of about 1-2 km wavelength, and crest length of at least 5 km, have been followed for up to about 25 km and, although largely parallel, they also branch and are noticeably sinuous. Near to the western end of the Mediterranean Ridge the structural grain turns round to the north (Fig. 1) and seems to terminate against a fault zone, which is close to the northern end of the Hellenic Arc⁴ and to the northern limit of earthquakes in the Ionian Basin⁵. Extending south-westwards from this point a new branch, with a well developed grain in similar style topography, has been discovered. This dies out just before reaching the faulted, 3,000 m high Malta Escarpment. The inverted 'Y' shaped junction between these two Ridges encloses two

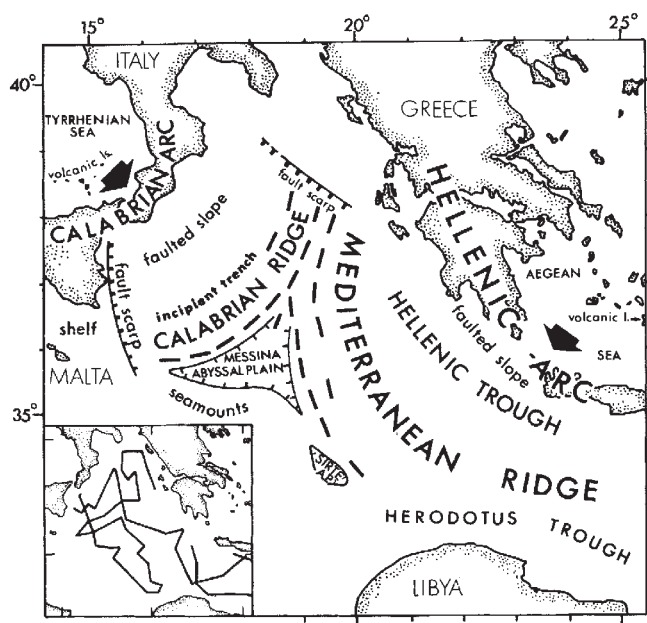


FIG. 1 Schematic diagram showing the inverted Y-shaped junction between the Calabrian and Mediterranean Ridges, together with the related structural elements bounding the Ionian Sea. The black bars provide representative indications of the grain of the ridges and troughs along the edges of the Messina abyssal plain, that were detected by long range side-scan sonar (Gloria). The inset figure gives the tracks along which Gloria sonographs were recorded, the line thickness about equalling the 14 km wide belt that was examined. The large arrows show the directions of inferred motion of the crust at the Hellenic and Calabrian Arcs with respect to the Ionian Sea.

sides of the triangular shaped Messina Abyssal Plain.

This western branch we call the Calabrian Ridge, as it lies essentially concentric with, and is probably related in origin to, the Calabrian Arc (Fig. 1). The Calabrian Ridge is not a pronounced topographic feature, so its prior lack of recognition is not surprising. Its landward side is defined on bathymetric charts by a series of local deeps⁶ marking the location of an incipient trench. An air gun profile extending from the western end of the Messina Abyssal Plain towards the toe of Italy shows a similar sequence across the Calabrian Ridge to that seen on crossings of the main Mediterranean Ridge. An acoustically transparent layer (Plio-Pleistocene, presumably) 600 m thick, assuming a two-way travel time of 2 km s⁻¹, overlies the 'M' reflector (Miocene evaporite sequence, presumably). At the abyssal plain both layers are only slightly disturbed, whereas on the Calabrian Ridge both the sea floor and the 'M' reflector are so broken that the latter appears to be diffuse. The narrow incipient trench (analogous to the series of deep trenches collectively known as the Hellenic Trough) has a flat floor underlain by up to 800 m of layered (and faulted) fill. Between this trench and the Italian coast alongslope tectonic trends are seen on sonographs, although without the distinctive texture of the Calabrian Ridge (as seen on sonographs and the air gun profile). This slope is not a depositional cone as previously thought⁷, although there are some downslope trending canyons cut into it. The similarity of the Calabrian and Hellenic Arcs is further emphasised by the extremely narrow continental shelves and by the similar distance between the trenches and the inner volcanic arcs. The Hellenic Arc is currently associated with frequent shallow and intermediate depth earthquake activity, and the Calabrian Arc more with deep focus activity^{4,8}, which may suggest that the former is currently in a vigorous stage of activity and the latter in a dying phase. Thus, although

the Calabrian Ridge need not necessarily further develop a pronounced relief, it nevertheless implies crustal shortening related to compression which is associated with the Benioff zone beneath the Calabrian Arc, probably as a result of south-easterly motion of the Tyrrhenian region. This is similar to the proposed relationship between the Mediterranean Ridge and the Hellenic Arc². The inverted Y-shaped junction is thus the combined result of compression between the two arcs, which in travelling outwards are impinging on the Messina Abyssal Plain from two sides, accounting for the triangular shape of the Plain, since the southern side is bounded by a seamount chain.

We thank colleagues for operating and maintaining Gloria and air gun equipment at sea.

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Received December 28, 1973.

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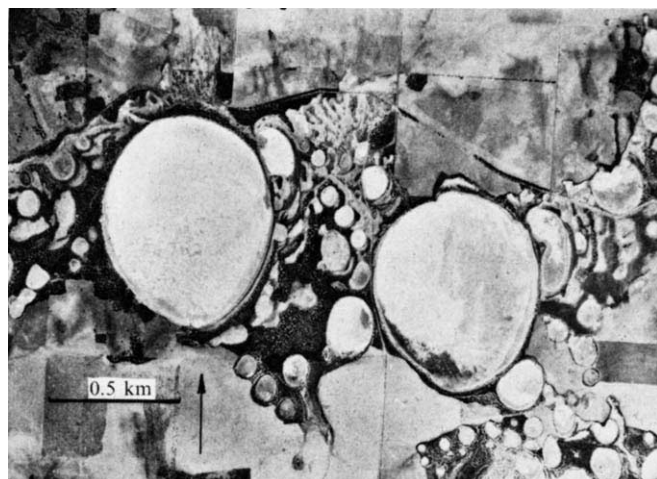


Fig. 1 Playa lakes near Moora, South Western Australia.

and closer to the west coast (Moora/Perenjori) many playa lake features are orientated slightly west of N (at a mean orientation of 347°). Inland long axis orientation is east of N and in the southern drainage systems (Corrigin, Hyden, Newdegate) mean long axis orientation is 33° east of north.

Hydrodynamic analysis of shaping processes in shallow lakes in unconsolidated sediment is well documented³⁻⁷. Such studies show that consistency of orientation, high ellipticity values and smooth curvature of shoreline are equilibrium forms of wave generated currents, reflecting zones of maximum mean current velocities around margins. Bruun³ indicated that maximum littoral drift occurs on downwind shores where angles between wave orthogonals and normals to shoreline are 50°. Minimum littoral drift occurs where these angles are 0°. For shorelines of limited length a cycloid equilibrium outline results which is orientated normal to dominant winds. Reversal of dominant winds produces two opposite shorelines of cycloid form forming a basin of elliptical shape⁴. In an effort to explain the data in Figs 2-4 we tested the applicability of this model derived from analysis of current flow on beaches and in permanent lakes in relation to playa features aperiodically flooded. Following the flooding of many local South-west Division playas during winter 1973, rhodamine B dye was used to

Development of Playa Lakes in South Western Australia

SEDIMENT composition and provenance studies in ephemeral drainage systems of South Western Australia have shown interesting regional variation in the size, shape and orientation of playa lakes.

A major continental divide separates two drainage divisions in South Western Australia¹. The South-west Division centres on the Avon and tributary systems, while the drier Eucla Division to the east is endoreic and contains many large playas. Here we describe the influence of winds on the morphology of playa lakes in the South-west Division, examples of which are shown in Fig. 1.

Figure 2 shows a size distribution plot for 600 playa lakes measured from aerial photo mosaics (1:63,360), twelve of which cover each topographic sheet (1:250,000) mentioned (Moora, Perenjori, and so on). The size range extends from features at the lower limit of measurement, 0.004 km² to several playa lakes over 100 km². The concentration around 0.05 km² reflects the presence of numerous small playa lakes in the South-west Division known locally as 'salt pans'. A characteristic of these playa lakes is the recurrence of certain shapes, commonly elliptical, obloid, circular and elongate. Figure 3 shows how the shape index E ($E = (L-W)/L$, where E =ellipticity, L =long axis length and W =short axis length²) varies within the same drainage systems. There is a significant concentration of values around $E=0.33$.

Regional contrast is evident in preferred long axis orientation measured against 'photo' north (Fig. 4). In the north

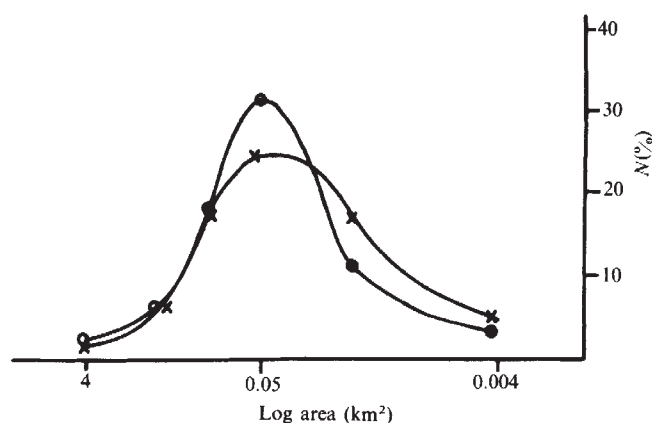


Fig. 2 Size distribution of six hundred playa lakes in South Western Australia measured from 1 mile to the inch air photograph mosaics. Place names refer to 1:250,000 topographic maps covered in this study. ○, Hyden, Corrigin, Newdegate; ×, Moora, Perenjori.

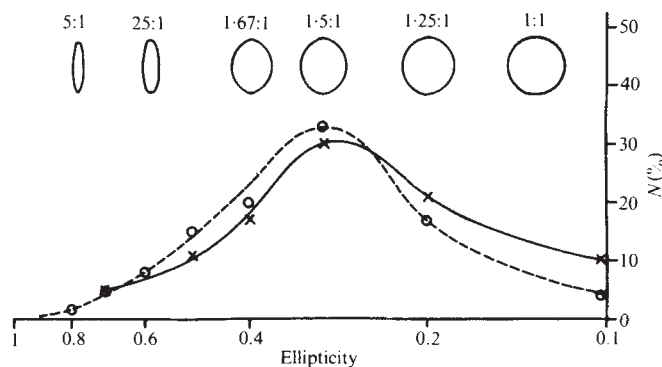


Fig. 3 Shape distribution of 600 playa lakes in South Western Australia measured from 1 mile to the inch air photograph mosaics. Place names refer to 1 : 250,000 topographic maps covered in this study. $\circ$, $\times$, As Fig. 2.

track and measure currents in about 10 cm of water during winds of velocities up to 900 cm s^{-1} . Fine colloidal streamers of sediment are agitated into suspension and moved by littoral currents. Patterns and velocities of flow agree in general with current measurements of Carson and Hussey⁴. Sorting analysis of sand from small sandy beach deposits around many playas also reflects velocity differentials.

In South Western Australia wind velocities and magnitudes have been measured since 1964, as two daily Beaufort Scale recordings over sixteen compass points. From this data averages for both twelve month and six winter months (May–October) periods were made for twenty centres in the South-west Division.

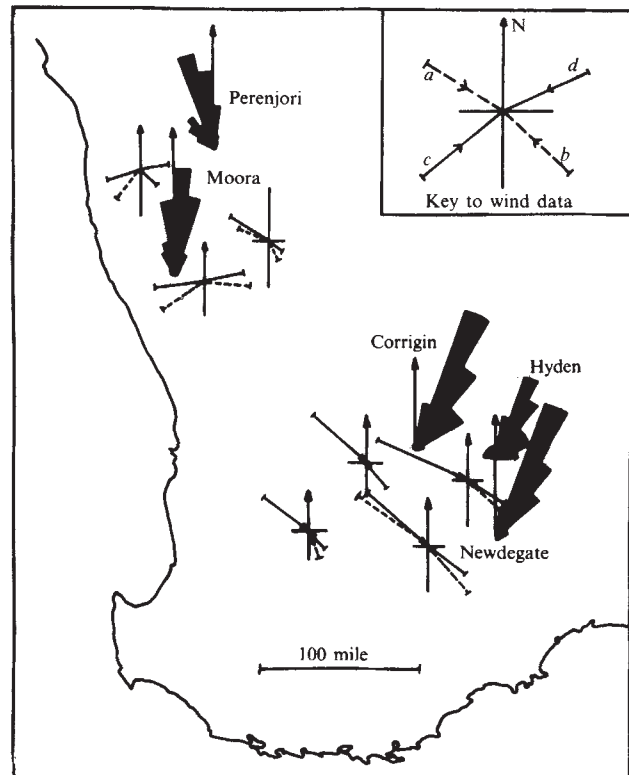


Fig. 4 Rosette diagrams showing long axis orientation of playa lakes in South Western Australia. Wind vector resultants about mean playa lake orientation are also shown for adjacent centres (a, Western component 12 month data; b, eastern component 12 month data; c, western component for 6 winter months; d, eastern component 6 winter months. Where 12 month and 6 winter month vectors are within 5° of each other only the winter vector is shown.)

It was argued that if local playa lakes had a sufficiently high flooding ratio⁸—the number of days they contain water each year/365—and if as indicated, the Bruun model is generally applicable, a correlation should exist between winter-wind resultants and playa lake orientation. Final resultants ought to parallel the mean short axis orientation in a particular region, and should be approximately opposed. Such correlations have been shown to exist in shallow lake systems elsewhere^{4,5}. In the areas investigated a 46° variation in regional long axis orientation is indicated and hence simple vector resolution about N–S as used by Carson and Hussey⁴ is pointless if the object is to test the above relationships. But when vector resolution is made about mean playa long axis orientation for a particular area, winter wind resultants remain approximately normal to long axis orientation and opposite each other. Resolutions about other directions do not give this result.

Figure 4 shows the regional long axis orientation of playa lakes in the South-west Drainage Division. Also shown are wind vector resolutions for selected sites made around the mean long axis orientation of nearby playa lakes. As indicated a sharp contrast is evident in both sets of data between the northern and southern parts of the division. Within the Moora/Perenjori set long axis orientation has a north-north-western alignment in the coastal examples, while inland long axis orientation swings east of north. In the southern part of the division (Corrigin, Hyden, Newdegate) inclination of long axis to the north-north-east is well developed, though an increasing variability is evident with increasing aridity (such as Hyden).

Wind vector resolution around the mean long axis orientation of local playa lakes consistently produces near normal resultants for winter winds. A greater variation in the corresponding twelve months data is observed. This close agreement of winter wind vector resultant and normals to long axis orientation occurs in both the north coastal and inland examples.

Studies of fluvial systems have encouraged the view that process and sedimentary medium interact to produce a continuum of drainage patterns⁹. Drainage features in ephemeral systems are an important set in such a continuum, comprising a variety of related forms.

The evidence described above suggests that playa lake features within the ephemeral drainages systems of the South-west Drainage Division display an equilibrium adjustment of shape, size and orientation to contemporary fluvioaeolian processes. In spite of aperiodicity of rain, flooding ratios are apparently high enough to shape and orientate playa lakes. The relative position and form of margin deposits, both beach and lunette, appear to be related to Bruun's³ maximum/minimum zones of current erosion and deposition, with subsequent movement by wind.

We thank Western Mining Corporation for financial support.

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Received October 15; revised November 30, 1973.

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Support for atmospheric origin of anomalous submillimetre wave spectral features

A RECENT letter in *Nature*¹ showed a feature at a frequency of 8 cm^{-1} in one of two spectra of the atmosphere. That the feature is an artefact follows from the authors' own statement that no true spectral observation was possible. The indication was found only in records from daylight hours and the proposal was made that it was caused by some solar process with an appropriate time variation coupling to the recording system in some unspecified way. We do not wish to comment on this process or on the authors' own evidence given to support it. But they also quote published results as giving further support to their hypothesis with the clear implication that features in the spectra of other workers may be due to the same kind of artefact. We contest this since it is easy to distinguish between a true spectral indication and an artefact, coming from temporal variations of source or absorption intensity, by varying the time rate of change of path difference given by the interferometer.

TABLE 1 Properties of spectra in Fig. 1

	Curve A	Curve B	Curve C
Precipitable water (mm)	0.85	0.85	1.84
Path length (m)	80	80	20.25
Temperature (K)	293	293	328
Resolution (cm^{-1})	0.25	0.25	0.5
Transmission value at 8 cm^{-1}	0.95	0.75	0.60

Baluteau *et al.* cite evidence (see ref 2) with the mistaken supposition that the results came from measurements using the Sun. It was clearly stated that spectra were obtained using an artificial source and a horizontal atmospheric path and, to add to the record, the spectra were recorded in the hours of darkness. The argument for this work sup-

porting their hypothesis is therefore invalid.

In stating that "effectively laboratory spectra" do not show any anomalous peak in this spectral region Baluteau *et al.* seem to have overlooked other work of ours (ref. 3). Though this gave somewhat lightweight evidence it seems to have been essentially correct and provided an important clue about anomalous absorption. New work which relates to it is shown in Fig. 1 curve c.

The topic of anomalous millimetre and submillimetre absorption is being closely studied at the Appleton Laboratory. The two observed spectra (Fig. 1 curves b and c; Table 1) which are taken from work which will later be published in full, support our view that anomalous absorption is an atmospheric phenomenon and that the molecular complexity of water vapour is an important, if not the only factor in explaining it.

This work is part of the programme of the Appleton Laboratory.

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Received November 12, 1973.

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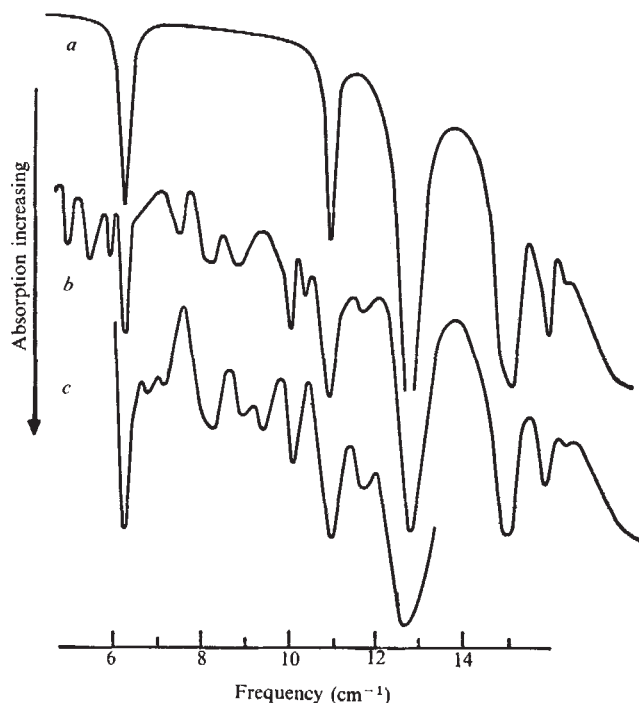


FIG. 1 Water vapour absorption spectra. a, Theoretical spectrum for monomeric water. b, Measured spectrum for a horizontal path in the atmosphere. c, Laboratory spectrum for water vapour alone. See Table 1.

Safety in the preparation of multimetallic catalysts

THERE is presently an extensive research effort in the field of catalysis concerned with alloy systems. Many researchers are preparing and characterising unsupported systems in order to study the metal phase in the absence of the support. A classical approach to doing this is that of simultaneous chemical reduction of the salts of a given alloy system in aqueous solution. Such reducing agents as hydrazine or sodium borohydride are frequently used. During the course of our work we found that the reduction of ruthenium salts with borohydride solutions gave a 'metal powder' which, when dried, violently exploded on touching it with water and sometimes by simple stimulation with a spatula. Under certain conditions a very unstable and poorly characterised 'ruthenium hydride' is apparently formed. A safer alternative technique is to use aqueous solutions of hydrazine for the chemical reduction of ruthenium-containing solutions.

Along these same lines, supported metal catalysts which contain gold should never be prepared by impregnation of a support with solutions which contain both gold salts and NH_4OH . The dried catalysts contain extremely shock-sensitive gold-nitrogen compounds which may explode with the lightest touch.

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Received December 19, 1973.

BIOLOGICAL SCIENCES

Subcellular Separation and Molecular Nature of Human Histocompatibility Antigens (HL-A)

HISTOCOMPATIBILITY antigens have been solubilised by various means¹, especially proteolytic digestion²⁻⁴, sonication⁵ and extraction with 3 M KCl⁶ or detergents⁷⁻⁸. Although detergents seem to solubilise the intact antigens these preparations have, until recently, proved difficult to fractionate further, whereas the products of the other procedures have proved amenable to purification but probably represent fragments of the membrane-bound antigen. This interpretation is based on the following arguments. First, solubilisation with 3 M KCl seems to depend on the presence of cell sap and was markedly reduced by protease inhibitors⁹. Second, in contrast to the detergent-solubilised antigen¹⁰, the other procedures yield non-aggregated products which, in general, possess similar molecular weights within the range 35,000–55,000¹⁰⁻¹². Third, mouse and human histocompatibility antigens obtained by papain digestion seem to be glycoproteins (3–8% carbohydrate), whereas the antigens solubilised from guinea pig and human tissues by sonication and 3 M KCl extraction have been reported to be non-glycosylated (less than 1% carbohydrate)¹². This dichotomy would be explicable if the latter procedures had caused degradation and the consequent separation of the carbohydrate from the molecule bearing the antigenic determinant.

If the above views are correct then conclusions on the molecular nature of histocompatibility antigens adduced from these non-detergent solubilised preparations are questionable. Solubilisation procedures relying on degradation also have other disadvantages. The yield of antigen is usually low, fragments are generally less immunogenic than the whole molecule and may induce a different spectrum of immune responses (for example, cellular as against humoral), and the products are not suitable for determining the antigen's mode of integration into the cell surface membrane. These disadvantages have led us to develop an alternative approach using a highly purified preparation of the surface membrane of a human lymphoblastoid cell line as the source of HL-A antigens, and solubilising the antigens completely with sodium deoxycholate. This detergent circumvents the difficulties associated with the strongly ionic detergents such as sodium dodecyl sulphate (SDS). It has also enabled separation of the HL-A antigens from the majority (90%) of the membrane proteins and the estimation of some of the molecular parameters of the apparently intact antigens. Similar approaches based upon the use of the non-ionic detergent, NP-40, have recently been reported^{8,10}. A com-

parison of the results reveals some interesting differences in the apparent molecular sizes of the antigens solubilised by deoxycholate and NP-40.

Antigenic activity was assessed by inhibition of fluorochromatic cytotoxicity¹³ using microtest plates (Searle Diagnostics, High Wycombe, Bucks.). The choice of this assay was based on its applicability to whole cells, subcellular fractions, and solubilised materials (Table 1), and its reproducibility ($\pm 20\%$ for three separate estimations for deoxycholate-solubilised membrane over one month). Antigen ($1 \mu\text{l}$) was incubated under mineral oil for 1 h at room temperature with $1 \mu\text{l}$ of antiserum before addition of fluorescein-labelled, typed, peripheral blood human lymphocytes as target cells. Quantitation was achieved by titrating several dilutions of the antigen against nine dilutions of antiserum; the endpoint for each antigen dilution was the antiserum dilution giving 50% killing of the target cells compared with the controls without antiserum. These endpoints were plotted according to Reif's modification of von Krogh's equation¹⁴; linear plots were obtained and the intercept on the y axis represented the amount of antigen giving 50% inhibition (ID_{50}).

Antigens of the LA series were assayed using sera 'Yochum' for (HL-A2); 'Dovey' and 'Gillespie' for HL-A1; while sera 'Dovey' 'P1422B', and 'P1305B' were used for HL-A8 and 'P1040B' for HL-A13 of the series 4 antigens. The antisera were rendered effectively monospecific by using appropriate target cells. The possibility that these sera detected antigens unique to BRI 8 cells was excluded by using a panel of typed peripheral blood lymphocytes to define the antisera specificities, and as target cells in the assay. It has been suggested previously that cytotoxicity is facilitated by a heterophile antibody present in the rabbit complement¹⁵; in this case, if BRI 8 cells absorbed this antibody or were anti-complementary by another mechanism, then non-specific inhibition would occur. This was ruled out by demonstrating that previous adsorption of the complement (10^7 BRI 8 cells per ml equivalent to 50 ID_{50} units in the assay, for 1 h at 4°C) had no discernible effect on the antigen titre. The problem of nonspecific killing of the target cells by the deoxycholate added with solubilised antigens was circumvented either by diluting the fractions until they were no longer cytolytic, by diluting the antiserum in a gross excess of extraneous protein (20% foetal calf serum) or by previous removal of the detergent using Amberlite XAD-2; in general, a combination of the first two procedures was used.

Although H-2 and HL-A antigens have been claimed to be associated with intracellular membranes, various results suggest that they are located mainly on the cell surface. As a result, a purified preparation of the surface membrane was chosen as the antigen source since, in this way, degradation by cell sap⁹ before or during solubilisation should be minimal.

BRI 8 cells, a diploid lymphoblastoid cell line derived from

Table 1 Recovery of HL-A2 antigenic activity on cell disruption, extraction of cells with 3 M KCl and solubilisation of the plasma membrane with 1% Na deoxycholate

Material	Initial HL-A2 activity* (ID_{50} total units)	Treatment	Recovered HL-A2 activity* (ID_{50} total units)	Recovery (%)
BRI 8 cells 8×10^9	7.3×10^6	Disruption †	7.3×10^6	100
BRI 8 cells 7.6×10^{10}	7.0×10^7	Disruption †	8.3×10^6	118
BRI 8 cells 2×10^9	1.8×10^6	Extraction 3 M KCl ‡	2.4×10^5	12
BRI 8 plasma membrane	6.7×10^5	Solubilisation by { Soluble	6.0×10^5	90
		Na deoxycholate § { Insoluble	3.4×10^3	1

* Assayed using serum 'Yochum'.

† As described for the preparation of plasma membrane (see text); these figures are for two different preparations.

‡ As described by Reisfeld *et al.*⁶.

§ Plasma membrane was incubated for 30 min at room temperature in 1% Na deoxycholate and then centrifuged at 100,000g for 1 h as described previously²⁹.

Table 2 Distribution of protein, HL-A2 antigenic activity and 5'-nucleotidase on fractionation of disrupted BRI 8 cells

Fraction	Protein (mg total)	5'-Nucleotidase activity		HL-A2-activity*		Ratio 5'-nucleotidase/ HL-A2 ($\times 10^4$)
		Total ($\mu\text{mol Pi/h}$)	Specific ($\mu\text{mol Pi/mg}$ protein/h)	Total (ID ₅₀)	Specific (ID ₅₀ /mg protein)	
Homogenate (4×10^{10} cells)	5,520	552	0.10	3.5×10^7	6.3×10^3	6.3
Pooled nuclear and mitochondrial	3,410	170	0.05	1.4×10^7	4.2×10^3	8.4
Microsomal			1.93		1.0×10^5	5.2
Supernatant	2,190	44	0.02	3.5×10^6	1.6×10^3	8.0
Endoplasmic reticulum †	54	49	0.91	—	—	—
Plasma membrane †	34	174	5.10	5.1×10^6	1.5×10^5	3.0
Unretarded ‡	6.3	0	0	0	0	—
Glycoprotein ‡	0.64	20.5	32.0	1.4×10^6	2.2×10^6	6.8

Fractionations were carried out as previously described (ref. 20 and M. J. Crumpton and D. Snary, in preparation) using differential centrifugation, sucrose density gradients and LcH columns.

* Assayed using serum 'Yochum' but similar results were obtained with serum 'Dovey'.

† Separated from the microsomal fraction by sucrose density gradient centrifugation (Fig. 1).

‡ Separated from deoxycholate-solubilised membrane (7.5 mg membrane protein) by chromatography on LcH-Sepharose (Fig. 2).

normal human peripheral blood [HL-A antigens: 1,2,8,13 (4a, 4b)], were purchased from Searle Diagnostics. Cells were grown in spinner culture in RPMI 1640 medium supplemented with 10% (v/v) foetal calf serum and were collected while growing logarithmically since membrane prepared from terminal cultures showed by polyacrylamide gel/SDS electrophoresis evidence of degradation. The cells collected were resuspended in Eagle's minimal essential medium (MEM) (5×10^7 to 2×10^8 cells per ml of MEM) and were disrupted at 0°C by extrusion at a rate of about 400 ml h^{-1} through a small orifice under a pressure of about 750 pound

inch² against a spring-loaded ball (developed from a design by Dr P. M. Wright). Under these conditions the cell number was reduced by about 50% and the viability, as measured by trypan blue exclusion, decreased from about 85% to 5%; electron microscopy revealed whole nuclei and cells denuded of their surface membrane. Table 1 shows that disruption was not associated with any significant change in the amount of HL-A2 antigen. This result agrees with that reported previously for H-2 antigens^{16,17} and is consistent with the view that histocompatibility antigens are expressed mainly, if not exclusively, at the cell surface.

The homogenate was fractionated as previously described¹⁸ by differential centrifugation (Table 2) and the microsomal fraction was subsequently purified by centrifuging on a continuous or discontinuous sucrose density gradient (Fig. 1). All operations were performed at 0°C and as quickly as possible to reduce degradation to a minimum. Addition of diisopropylfluorophosphonate in isopropanol to the microsomal fraction ($1\text{ }\mu\text{l}$ of a molar solution per ml) was discontinued when it was noticed that it caused aggregation of membrane vesicles. The most notable feature of the results shown in Table 2 is the very close similarity in distribution of HL-A2 antigen and the plasma membrane marker, 5'-nucleotidase, amongst the various subcellular fractions. This close association is confirmed in Fig. 1 which shows that these activities were distributed in an identical manner on sucrose density gradient centrifugation of the microsomal fraction. Furthermore, both activities were concentrated in the leading edge of the protein peak with a sucrose density of 1.14 g cm^{-3} which is characteristic of plasma membrane¹⁸ and neither activity was present to any significant extent in the band of higher sucrose density that possesses the endoplasmic reticulum marker, glucose 6-phosphatase. These results confirm and extend those reported recently by Wilson and Amos¹⁹ and emphasise the more or less exclusive association of HL-A antigens with the plasma membrane.

The plasma membrane fraction consisted predominantly of smooth membrane vesicles which according to various morphological and biochemical criteria were not contaminated by significant amounts of nuclei, mitochondria, endoplasmic reticulum, lysosomes, ribosomes or cytosol (M. J. Crumpton and D. Snary, in preparation). The purified membrane was suspended in 10 mM Tris-HCl buffer, pH 7.4, or dissolved in 1% Na deoxycholate in 10 mM Tris-HCl buffer, pH 8.2 and stored at -70°C . Alternatively, small aliquots ($10\text{--}40\text{ }\mu\text{l}$) of membrane suspension, solubilised membrane or 3 M KCl extracts⁶ were stored in plastic straws plugged with plasticine in liquid N_2 (ref. 20). Storage of the membrane suspension at 0°C or successive freezing and thawing gave marked reductions in HL-A activity and in solubility in deoxycholate. These changes were probably

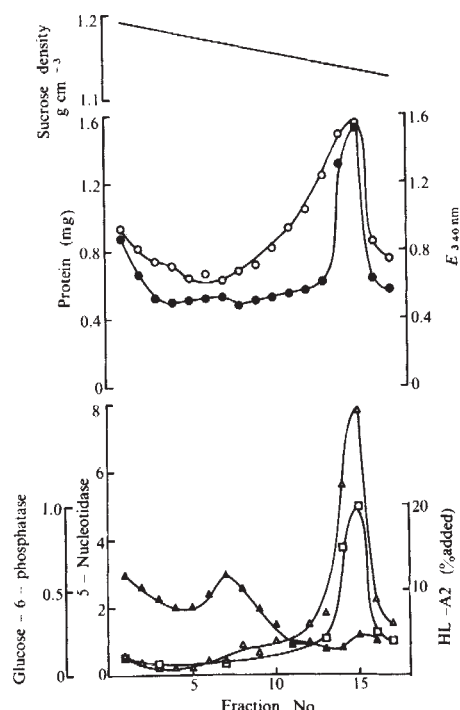


Fig. 1 Fractionation of microsomal pellet by sucrose density gradient centrifugation. The microsomal fraction was resuspended in 3.5 ml of 10 mM-Tris-HCl buffer, pH 7.4, by using a tight-fitting homogeniser and 1 ml samples were layered onto continuous sucrose gradients [8 ml of 25% (w/v) against 8 ml of 42%]. After centrifuging at 22,000 r.p.m. for 18 h in a SW27 rotor, 1 ml fractions were removed from just above the small pellet by using a Perplex pump. Samples were analysed for sucrose density, turbidity ($\bullet$, $E_{340\text{nm}}$), protein ($\circ$), 5'-nucleotidase (Δ , $\mu\text{mol Pi per mg protein per h}$), glucose 6-phosphatase ($\blacktriangle$, $\mu\text{mol Pi per mg protein per h}$) and HL-A2 activity ($\square$).

due to degradation since on polyacrylamide gel/SDS electrophoresis the aged membrane gave more intensely staining low molecular weight bands and fewer high molecular weight bands than the fresh membrane. In this case, in view of the membranes' high degree of purity it seems likely that this degradation is an intrinsic property of the membrane. In contrast, the HL-A activity of solutions of the membrane glycoproteins (see below) in deoxycholate was stable at -20°C and to successive freeze-thawing.

Recently a method was described for the isolation of membrane glycoproteins by means of affinity chromatography of deoxycholate-solubilised membrane on *Lens culinaris* phytohaemagglutinin (LcH) covalently attached to Sepharose^{21,22}. LcH possesses an antibody-like specificity for glucose, mannose and sterically-related sugar residues²³. If, as seems likely, HL-A antigens are glycoproteins then this method should be applicable to their separation from the membrane lipids and non-glycosylated proteins.

The purified plasma membrane was solubilised in 1% Na deoxycholate (4 mg of membrane protein ml^{-1}) by incubating for 30 min at room temperature followed by centrifuging at 100,000g for 1 h. The supernatant fraction contained about 90% of the membrane protein, 5'-nucleotidase and HL-A2 antigenic activity. In view of the almost complete recovery of HL-A2 activity on solubilisation (Table 1) it seems, in contrast to previous work using NP-40⁸, that serum 'Yochem' recognises membrane-bound and solubilised antigen equally well. The fractionation of the deoxycholate-solubilised membrane using LcH-Sepharose is illustrated in Fig. 2. About 10% of the added protein was bound to the column and was subsequently eluted with methyl- α -D-mannopyranoside. The eluted fraction possessed the majority (87%) of the added HL-A2 activity (Table 2) and all of the known membrane glycoprotein marker, 5'-nucleotidase²⁴. In contrast, the unretarded material (fraction No. 4, Fig. 2) contained no detectable 5'-nucleotidase activity and also failed to inhibit cytotoxicity due to serum 'Yochem' at a dilution

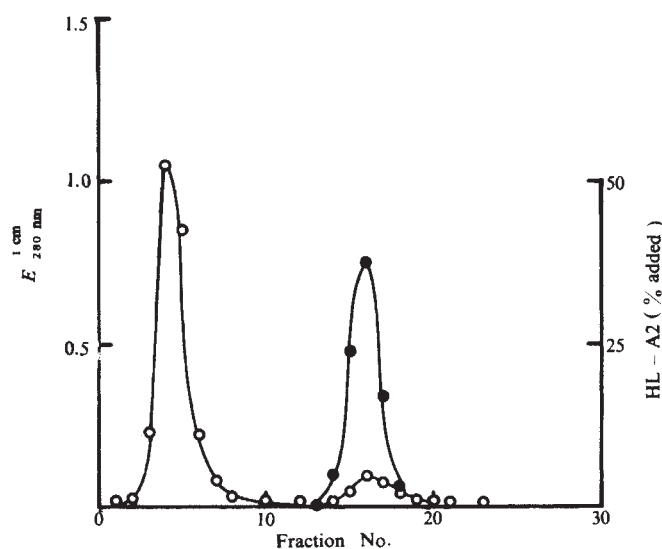


Fig. 2 Fractionation of BRI 8 plasma membrane using LcH-Sepharose. Membrane containing 8 mg of protein was incubated for 30 min at room temperature with 2 ml of 1% Na deoxycholate in 10 mM Tris-HCl buffer, pH 8.2, and centrifuged at 100,000g for 1 h. The supernatant (7.5 mg of protein) containing about 90% of the HL-A and 5'-nucleotidase activities was eluted from a 10 ml column of LcH-Sepharose with 1% deoxycholate until the extinction of the eluate at 280 nm returned to the base line. The column was then washed (fraction No. 12) with 2% (w/v) methyl- α -D-mannopyranoside in 1% deoxycholate. Fractions (2.7 ml) were assayed for protein (○) and for HL-A2 activity (●). HL-A activities corresponding to less than 0.8% of the activity added to the column would not have been detected under the assay conditions used.

of 1:5 or at 1:1 after removal of deoxycholate using Amberlite XAD-2. In view of the distribution of 5'-nucleotidase activity and the known specificity of LcH²³, it was concluded that all the HL-A2 antigen was glycosylated. This conclusion is contrary to that derived from the analysis of HL-A antigens solubilised by sonication or 3 M KCl extraction¹⁹ and is consistent with the suggestion that the latter preparations had undergone partial degradation.

The yield of HL-A2 activity in the glycoprotein fraction relative to that of the cell homogenate varied from 15 to 40% and the overall purification was about 350-fold; the variation in yield depended solely upon the recovery of plasma membrane which was apparently inversely related to the number of cells used, in that the higher yields of membrane were invariably obtained using smaller numbers (8×10^9) of cells. If it is assumed that approximately 1.0% of the membrane protein is HL-A antigen (about 4×10^6 mol per BRI 8 cell) then 10% of the glycoprotein fraction is HL-A. Comparisons of the above yield with those achieved previously for the same degree of purification are difficult due to lack of information. The present yield of membrane-bound antigen would, however, seem to be comparable with the highest recovery (about 16%) reported for the papain-solubilised antigen⁴ and, in some cases, to be noticeably superior (a yield of 1-2% for 350-fold purified papain-solubilised H-2 antigens³). Although higher recoveries have been claimed for 3 M KCl-extracted antigens⁶, this technique gave yields of 12% only (Table 1) in the present study.

Preliminary attempts to exploit lectins with different carbohydrate specificities to sub-fractionate the glycoproteins further have met with limited success. For example, *Axinella polyplodes* agglutinin (M. J. Crumpton and A. P. MacLennan, in preparation), which is specific for D-galactose residues, absorbed 4% of the membrane protein (that is, about 40% of the glycoprotein fraction) but none of the HL-A2 activity.

The molecular sizes of the membrane-bound HL-A antigens were estimated by sucrose density gradient centrifugation²⁵ of deoxycholate-solubilised BRI 8 plasma membrane in the presence of 1% sodium deoxycholate. The HL-A2 activity was distributed in an essentially symmetrical peak; the small pellet contained no detectable HL-A activity (Fig. 3). A comparison of the width of the peak with that of the membrane 5'-nucleotidase (Fig. 3) or a marker protein, aldolase, suggested however, that the HL-A2 antigen was slightly polydisperse. $S_{20,w}$ values were calculated relative to those of typical, water-soluble, globular proteins (such as, aldolase, bovine serum albumin), and are based on the assumption that the HL-A antigens and marker proteins possess similar partial specific volumes and molecular shapes in deoxycholate; this assumption is only valid if both groups of molecules do not bind the detergent or if they bind similar amounts (see below). The results for the reduced and unreduced, membrane-bound series LA and 4 antigens are summarised in Table 3; the values for the papain-solubilised HL-A antigens in deoxycholate are included for comparison.

The above results are notable for a number of reasons. First, the series LA and 4 activities sedimented at slightly different rates as judged using various anti-LA and 4 sera. This difference could be due to for example, series 4 antigens not being more readily degraded than LA antigens, or LA and 4 antigens having similar molecular sizes but binding different amounts of deoxycholate (that is, LA antigens may be integrated more deeply into the membrane lipid bilayer). Perhaps the simplest explanation, however, is that the LA antigens have a larger molecular size than the 4 antigens. This explanation is consistent with the report that in the mouse the antigenic specificities governed by the H-2K and H-2D genetic regions are expressed on independent molecules with slightly different molecular weights (44,000 and 39,000 respectively)¹⁰.

Second, the $S_{20,w}$ value for papain-solubilised HL-A

antigens in deoxycholate (3.68S) agrees closely with that determined for papain-solubilised H2 antigens in the absence of detergent (3.6S)¹¹. This agrees with the report that water-soluble proteins (the marker proteins and papain fragments) bind little or no deoxycholate²⁶.

Table 3 Comparison of $S_{20,w}$ of membrane-bound and papain-solubilised HL-A antigens of the LA and 4 series in 1% Na Deoxycholate

Material	$S_{20,w}$ * of HL-A antigens LA: series † 4 series ‡	
Deoxycholate-solubilised BRI 8 plasma membrane	5.15	4.55
Papain-solubilised§	3.68	3.68
Mercurioethanol treated deoxycholate-solubilised BRI 8 plasma membrane	5.24	4.35

* Estimated by sucrose density gradient centrifugation, as described by Martin and Ames²⁵, relative to the sedimentation of aldolase and bovine serum albumin under identical conditions; experimental details are given in the legend of Fig. 3. The figures in the table were obtained from separate tubes in a single centrifuge. Determinations of the $S_{20,w}$ for the LA series in 4 separate experiments were within range of 0.05 and two determinations for the 4 series agreed, to within experimental error (<0.05).

† Estimated using sera 'Yochum' and 'Dovey'.

‡ Estimated using 'Dovey', 'P1422B', 'P1305B' and 'P1040 B'.

§ A mixture of HL-A2 and HL-A7 obtained from separate spleens by papain digestion in the presence of 5 mM cysteine⁷ and donated by Dr A. R. Sanderson.

|| Membrane solubilized and centrifuged under reducing conditions in the presence of 2-mercaptoethanol.

Third, the deoxycholate solubilised HL-A antigens sedimented very much faster than the papain-solubilised material.

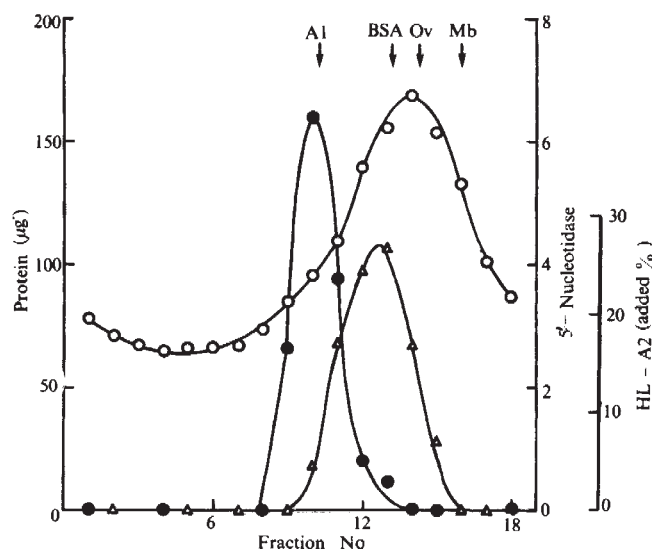


Fig. 3 Sucrose density gradient centrifugation of membrane-bound HL-A2 antigen dissolved in 1% Na deoxycholate. BRI 8 plasma membrane (28 mg of protein) was incubated for 30 min at room temperature with 7 ml of 1% Na deoxycholate in 10 mM Tris-HCl buffer, pH 8.2 and 1 ml of the mixture was layered onto a linear sucrose gradient [5 ml of 9% (w/v) against 5 ml of 28%] containing 1% Na deoxycholate. Marker proteins [aldolase (A1) 7.5S; bovine serum albumin (BSA) 4.41S; ovalbumin (Ov) 3.55S; sperm whale myoglobin (Mb) 2.08S] were added to identical gradients. The gradients were centrifuged at 4°C in a Spinco SW 41 rotor for 48 h at 38,000 r.p.m. (180 g_w). Timed fractions (0.67 ml) were removed from the bottom of the tube using a Perplex pump. Samples were assayed for protein (○), 5'-nucleotidase (●, μ mol Pi per mg protein per h) and HL-A2 activity (△) using serum 'Yochum'; serum 'Dovey' gave an identical distribution of HL-A1 activity. The small pellet at the bottom of the tube contained no detectable HL-A2 activity (see also Table 1).

This difference was not due to the reducing conditions (5 mM cysteine) used during papain digestion since treatment of the solubilised membranes with 2-mercaptoethanol had no discernible effect on the antigens' rates of sedimentation. Alternatively, it seems reasonable to assume that the difference is related to the presence in the membrane-bound antigens of a hydrophobic region which is necessary for insertion of the antigens into the membrane and which is detached during solubilisation by proteolysis (compare the influenza virus membrane glycoproteins^{27,28}). The actual difference in molecular size between the membrane-bound and papain-solubilised antigens is impossible to assess without knowing whether the whole antigen binds deoxycholate and, if so, how much. The results of Helenius and Simons²⁶ using erythrocyte membrane proteins as a model predict that the HL-A antigens should bind about 0.38 g of deoxycholate per g protein. In this case their rates of sedimentation would correspond to molecular weights of about 54,500 and 45,200 for the LA and 4 series respectively. On the other hand, it is questionable whether the HL-A antigens actually bind significant amounts of deoxycholate; thus, they failed to form the very large aggregates detected by Helenius and Simons²⁶. Also, not all membrane proteins apparently bind deoxycholate; for instance, the membrane 5'-nucleotidase behaves in deoxycholate (Fig. 3) as if it had a molecular weight of about 160,000 which corresponds closely to that derived for the mouse liver enzymes²⁴. In this case the molecular sizes of the LA and 4 antigens (88,000 and 73,000 respectively) are in close agreement with that reported for the 'dimeric' H-2 antigen (molecular weight 88,000) detected by polyacrylamide/SDS electrophoresis of NP-40 solubilised material¹⁰.

Fourth, the apparent molecular sizes of the deoxycholate-solubilised HL-A antigens were smaller than those reported previously for intact HL-A and H-2 antigens solubilised in NP 40 (200,000 and 380,000 respectively^{8,10}). Although the antigens may exist in the membrane as aggregates¹⁰ which were dissociated by deoxycholate, this seems unlikely since deoxycholate failed to cause any detectable dissociation of non-covalently bonded, multi-subunit proteins such as aldolase and haemoglobin (M. J. Crumpton and M. J. H. in preparation). The difference is probably related to the different properties of the detergents (such as micelle size) and possibly expresses different extents of binding. If this interpretation is correct then the functional membrane unit of H-2 and HL-A antigens may be a non-covalently bonded dimer with a molecular weight of about 90,000. The partially purified material has been used for the preparation of antisera in C₃H and Swiss-Webster mice. Preliminary results indicate that cytotoxic antisera can be made against the glycoproteins but not against the proteins from the BRI 8 membranes, and suggest that this material may prove very useful for raising HL-A antisera.

This study incorporates four significant observations. First HL-A antigens are located mainly, if not exclusively, in the cell surface membrane. Secondly, all the plasma membrane HL-A antigens are glycosylated and may be separated from the non-glycosylated proteins by affinity chromatography of deoxycholate-solubilised membrane on a column of LcH-Sepharose. Third, the molecular size of the series 4 antigens in deoxycholate is smaller than that of the LA series. Fourth, the intact HL-A antigens are larger than the papain-solubilised fragments. Although the actual molecular size of the membrane-bound antigens is at present uncertain, some results suggest it is about 90,000.

The methods used in this study have avoided the technical difficulties associated with the isolation of water-insoluble membrane proteins in a biologically active form and should enable the separation of the whole antigens in amounts sufficient for structural studies.

The work reported here was supported in part by a grant from the Medical Research Council (to the Genetics Labora-

tory, Oxford); P. G. and M. J. H. are recipients of Research Scholarships from the Medical Research Council.

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Received October 5, 1973.

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Human Normoblast A Antigen seen by Immunoelectron Microscopy

MEMBRANE antigens are extensively studied on different mammalian cell lines. Antigens of the erythrocyte series are often chemically well defined heterosaccharide determinants¹, the subject of numerous studies, most of them restricted to the mature circulating erythrocytes. There are only a few reports dealing with the detection of blood group antigens on erythrocyte precursor cells²⁻⁵. Yunis and Yunis² gave strong evidence of the presence of A, B and H alloantigens on human normoblasts, mostly by indirect agglutination methods involving living bone marrow cells.

Here we report immunoelectron microscopy observation of A antigen on human normoblasts using peroxidase-labelled antibodies which allow detection of surface antigens on separated cells. A preliminary report has appeared⁶ in which A antigen was detected by this method on circulating normal erythrocytes; a heavy labelling of A₁ red cells was found in most cases, and A₂ cells exhibited marked variations of antibody binding capacity.

Bone marrow was obtained by iliac puncture from four normal individuals (three A₁, one A₂). Aspirates were collected in Hank's heparinised solution (Liquemine Roche) and bone marrow particles isolated and dissociated by pipetting in Hank's. Nucleated cells were separated from fat droplets by several washes and centrifugation for 5 min at 200g. They were then fixed with phosphate-buffered glutaraldehyde (T.A.A.B. Laboratories) 1.25% for 30 min in the cold, and kept in buffer until following steps.

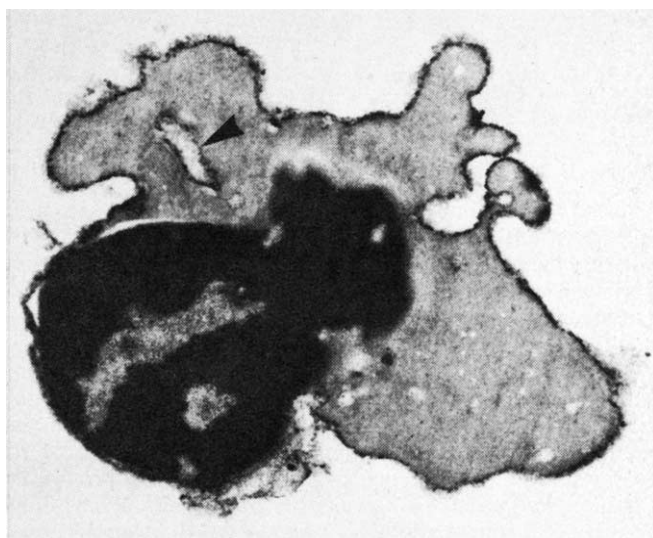


Fig. 1 Orthochromatic normoblast with nucleus in the way of expulsion. This motile cells has irregular borders, with a continuous labelling including the thin perinuclear cytoplasmic corona. Arrow, cross section of a membrane invagination, also labelled. Staining with uranyl acetate and lead citrate ($\times 7,600$).

The antiserum used was a human alloantiserum with an agglutination titre of 1:256 of A₁ cells which decreased to 1:2 after adsorption on A₂ cells. Activity was due mostly to IgM as the titre decreased to 1:8 after 2-mercaptoethanol treatment⁷. Monospecific anti-human IgM antibodies were prepared in rabbits. Purification was carried out by elution from IgM and cross reaction with IgG solid immuno-adsorbents⁸. Human IgM and IgG were purified by previously described methods^{9,10}. Purified rabbit anti-human IgM antibodies were conjugated with horse radish peroxidase (Sigma Laboratories) by a two step method¹¹.

Fixed cells were mixed with anti-A serum (3% cell suspension in 1:2 serum dilution) for 90 min at room temperature, extensively washed, and fixed again with glutaraldehyde for 10 min. After washing, sensitised cells were incubated with anti-IgM antibodies (1 mg ml⁻¹) for 1 h, washed and briefly fixed. Immunocytochemical staining was carried out by a 30 min incubation in the usual mixture of DAB and H₂O₂¹². Cells were then post-fixed with a 1% osmium tetroxide, dehydrated and embedded in Epon.

Specificity of labelling was ensured by the following controls: the treatment of group O and B bone-marrow cells; the omission of anti-A antibodies; the selective inhibition of labelling by non-conjugated anti-IgM; the use of normal conjugated rabbit IgG instead of anti-IgM and the incuba-

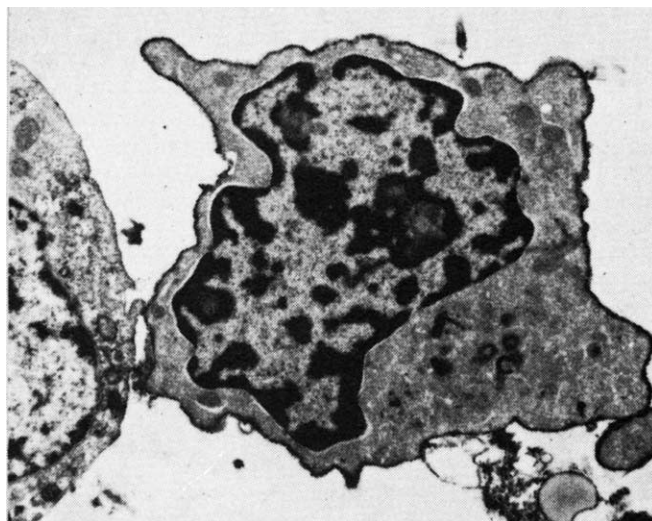


Fig. 2 Basophilic normoblast. The nucleus contains small clumps of heterochromatin. c, Two centrioles. The contiguous granulocyte precursor is not labelled. Staining with uranyl acetate and lead citrate ($\times 7,600$).

tion of cells with peroxidase alone.

A dense and continuous dark labelling was seen in contact with the membrane of all normoblasts, surrounding the whole perimeter of the cell, including rhopheocytosis invaginations. No significant variations of the thickness of the dark layer could be seen among normoblasts of a single bone marrow. A_2 normoblasts could exhibit slight variations in the labelling intensity, however, which had no relation to the maturation stage. Precise identification of the differentiation stage was made according to nuclear and cytoplasmic ultrastructural features¹⁸. Thus, all recognisable stages including pronormoblasts seemed to have a high density of antigen. Recognition of pronormoblasts may be difficult even at the ultrastructural level¹⁴ but the presence of ferritin-containing vesicles resulting from rhopheocytosis, revealed by high power views of these labelled undifferentiated blast cells, allowed a firm identification.

Occasionally a thin and limited labelling of the membrane of granulocytic and monocytic cells could be seen, sometimes in close contact with labelled normoblasts. Although the possibility of a 'false' labelling of these contiguous cells by 'flowing' of the reaction product, as already discussed by others¹⁵, cannot be excluded, this seems improbable as many other contiguous cells did not exhibit labelling at all. Finally, this faint positive reaction could result from the presence of A antigen on these non-erythrocytic cells, as already indicated by other methods¹⁶, but this requires further investigation. In addition, some lymphocytes and plasma cells were labelled and this was presumably due to the detection of surface IgM, as they were found in controls when anti-A was omitted as well as when O and B cells were tested.

The labelling of normoblasts appeared as a continuous layer of dense product, as in circulating erythrocytes⁵. No discontinuity was observed, suggesting a periodic pattern of A sites, and this is at variance with other studies using ferritin-labelled antibodies^{5,17}. Reasons for these discrepancies may be of a technical nature, as discussed elsewhere¹⁸.

Thus, our observations confirm and extend previous data from Yunis and Yunis² by demonstrating at the ultrastructural level the presence of A antigen on erythrocyte precursor cells. Indirect evidence of the presence of erythrocyte antigens on early precursors was also reported in mice³. On the other hand, some data on animals indicates that the appearance of antigen sites is in relation to the maturation of normoblasts, with detectable and reactive sites at later stages of this process^{4,5}. Although not quantitative, our findings clearly show, at least for human alloantigens, that such receptors are already numerous at the very early stage of erythrocyte differentiation.

We thank the Centre Departemental de Transfusion Sanguine du Val de Marne and the Fondation pour la Recherche Médicale Française for support.

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Received October 19, 1973.

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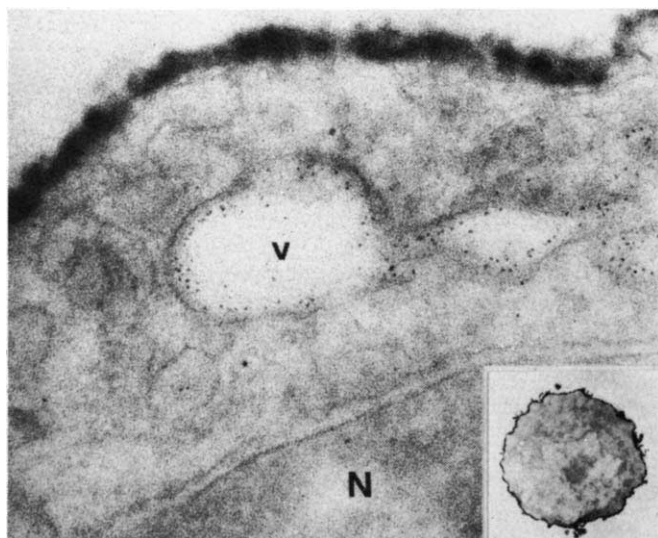


Fig. 3 Inset, heavily labelled pronormoblast. Large nucleolus is visible ($\times 2,300$). High magnification showing the membrane labelling, and ferritin molecules in micropinocytotic vesicles (v); these rhopheocytosis vesicles result from invagination of the outer membrane, previous to treatment of the cells and thus are not labelled. N, Nucleus ($\times 91,500$).

Intracellular Location of Specific Antibodies Reacting with Human Lymphocytes

THE concept of the continuous renewal of the plasma membrane of a cell has been well supported by studies on the rate at which histocompatibility antigens on the surface of the cell are replaced¹⁻⁴. Thus, if HL-A or H-2 antigens are removed by treatment with proteolytic enzymes, about 6 h elapse before the cell resynthesises them and they are again detectable with the appropriate antisera. When lymphocytes are incubated with a labelled anti-HL-A serum, label is gradually released during the next 6-8 h and the complement-dependent cytotoxic effects of an anti-HL-A serum are lost in a similar way; we call this 'escape from sensitisation'. This suggests that the turnover of the membrane of a cell may be primarily responsible for the ability of the cell to deal with antibodies binding to it, but the mechanisms involved are by no means clear. It has been suggested that antibody could either be released from the surface of the cell as an antigen-antibody complex or that it might be internalised, catabolised and then released, but neither of these ideas has been fully investigated. The early fate of fluorescent material has however been extensively studied^{5,6,7}.

Some subcellular fractions of the cell are known to carry the same type of histocompatibility antigens as those found on the plasma membrane⁸⁻¹¹, but it is not known whether there is any relationship between these internal antigens and those on the surface of the cell or whether they play any part in the way in which the cell deals with anti-HL-A antibodies. By following the fate of ¹²⁵I-labelled antibodies we thought that it might be possible to study these problems and we have therefore attempted to examine both the role of these internal antigens and the fate of the antigen-antibody complex formed at the cell surface.

Monospecific HL-A2 and HL-A8 antisera obtained from patients with choriocarcinoma and an antilymphocyte serum which was obtained by repeated injection of a rat with human lymphocytes were used, and were labelled in the manner described by Hunter and Greenwood¹².

The results of the two different methods for measuring the loss of antibodies are compared in Fig. 1. Lymphocytes from

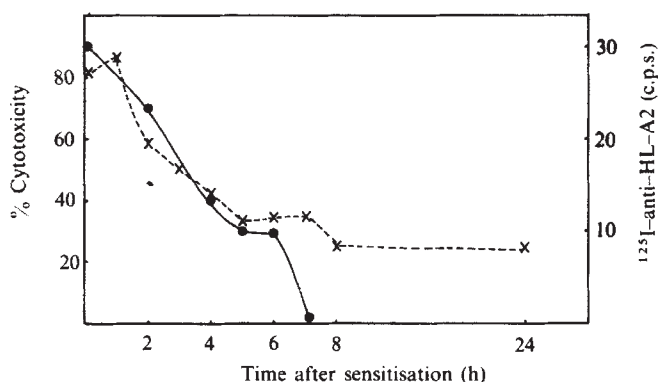


Fig. 1 Rate of loss of labelled anti-HL-A2 antibody (×...×) and loss of complement-dependent cytotoxicity (●—●) from lymphocytes of an HL-A2 individual. Antiserum was labelled in the manner described by Hunter and Greenwood¹², and cells were maintained in Eagle's MEM + 10% foetal calf serum after a 30 min sensitisation. Complement-dependent cytotoxicity was determined in the way described by Miyajima *et al.*⁴ except that sensitisation was carried out at 37° C.

an HL-A2 individual were sensitised by incubating them at 37° C for a short period (30 min) with a limited amount of an HL-A2 antiserum. After washing the cells to remove excess antibody, the complement dependent lytic activity was determined at hourly intervals. Lymphocytes carrying the same

histocompatibility antigen were treated in a similar way with ¹²⁵I-labelled antiserum so that the rate of loss of label could be compared with the rate at which the cytotoxic effects of antibody disappeared. Despite the fact that the two curves are very similar, it is evident that when all the cytotoxic activity of the serum has been lost, there is still a proportion of labelled material which is retained by the cells.

Table 1 Amount of ¹²⁵I-labelled anti-HL-A2 antibody binding to equal numbers of cells or nuclei* at 37° C

	HL-A2 individual	c.p.s. Non-HL-A2 individual
Cells	299.4 ± 45.2	20.2 ± 10.9
Nuclei	2,303.8 ± 99.3	4.4 ± 0.9

* 2×10^6 ml⁻¹.

The results of autoradiography and cell fractionation show that this residual antibody is no longer associated with the plasma membrane and escape seems to involve the intracellular relocation of antibody or antigen-antibody complex. This might account for the apparent inactivity of antibody retained by the cell, for once it has been removed from the plasma membrane complement-dependent cytotoxicity is no longer expressed.

Figure 2 shows that the labelled antibody which binds to the surface of cells is first processed through the patching and capping mechanism which has now become well established, but the step which follows this internalisation leads to the deposition of antibody in or around the nucleus.

Cell fractionation studies, Fig. 3, confirmed these observations and they also showed that label is lost from the cytoplasmic

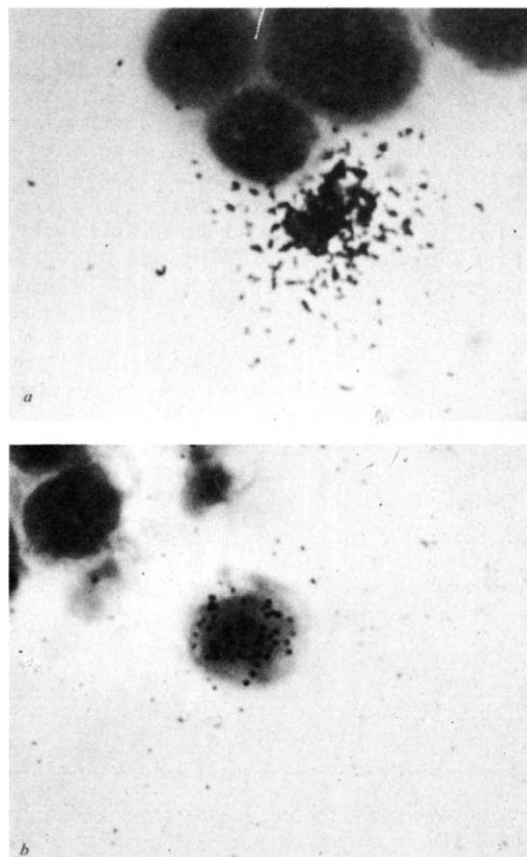


Fig. 2 Autoradiography of cells treated with ¹²⁵I-HL-A2. a, Cells immediately after sensitisation; b, 24 h after sensitisation.

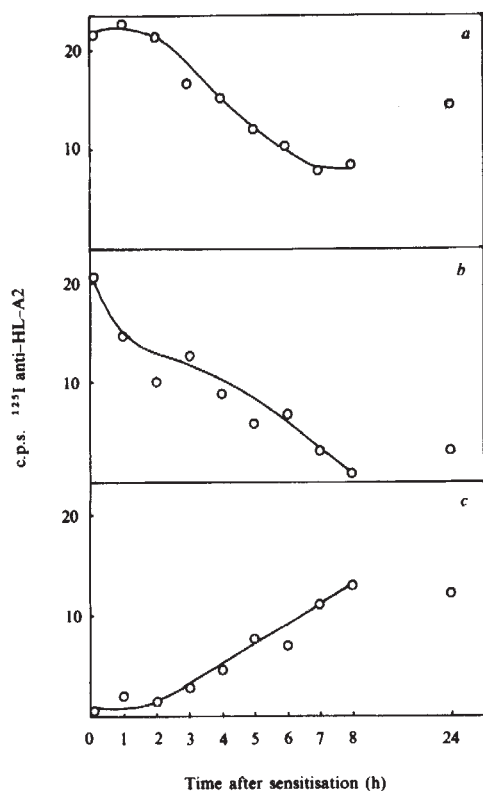


Fig. 3 ¹²⁵I-anti-HL-A2 in various cell fractions at intervals after sensitisation. *a*, Cells; *b*, cytoplasmic and, *c*, nuclear fractions prepared in the way described by Pegrum *et al.*¹³, cytoplasmic fractions being collected as washings after deoxycholate and Nonidet P40 treatment.

fraction at around 8 h. Escape from complement-dependent sensitisation therefore seems to be complete when antibody has been removed from the cytoplasm of the cell.

Albert and Davies¹¹ have shown that the nuclear membranes of cells carry the same histocompatibility antigens as those found on the external membrane. We also found that nuclear preparations carry HL-A antigens, for ¹²⁵I-anti-HL-A binds both to isolated lymphocyte nuclei and to whole cells. In fact, from the results shown in Table 1, it seems that nuclear preparations have a higher affinity for anti-HL-A antibodies than intact cells. This might suggest that the label we found in the nucleus after cell fractionation is the result of uptake during the extraction process. The autoradiographs, however, provide convincing evidence that antibodies are present in the different regions of the cell during the course of escape. We also observed that label could only be detected in the cytoplasmic fraction during the first few hours after sensitisation when the amount of antiserum binding to the cell was at its highest.

Table 2 Amounts of ¹²⁵I-labelled anti-HL-A8 antibody in various cell fractions at daily intervals after sensitisation at 37° C

	Initial	24 h	c.p.m. 48 h	72 h	96 h
Cells	1,097.5	492.0	681.0	549.3	620.5
Cytoplasm	1,220.3	60.6	126.9	80.7	9.75
Nuclei	120.7	626.3	672.0	693.2	645.2

In theory, it should be possible to determine the rate of turnover of HL-A antigens in the nucleus or on the nuclear membrane by following the fate of the ¹²⁵I localised in this region. The amounts in the various cell fractions were therefore assessed at daily intervals for some time after most of the antibody had been lost during escape and the results of the experiment are shown in Table 2. Even after 4 d, the amount of

labelled material in the nuclear fraction was at a relatively high level, the values being practically the same as those obtained from cells which had recently escaped sensitisation. Although this suggested that the nuclear membrane was very stable the same kind of results were not always obtained when the experiments were repeated with an anti-lymphocyte serum (ALS). We found that label only remained in the nucleus if the amount of antiserum used to treat the cells was kept to very low levels. The addition of anti-lymphocyte serum in quantities high enough to induce blastogenesis caused label to be released from both nucleus and the whole cell at a time which coincided with DNA synthesis; that is at about 72 h (Fig. 4). It is known that anti-HL-A serum is also capable of stimulating mitosis in lymphocytes and when cells were maintained in medium containing high levels of an anti-HL-A8 DNA synthesis was initiated. By first treating cells with labelled serum and then incubating them in medium containing greater amounts of the same cold antiserum, it could be shown that anti-HL-A is dealt with in a similar way to ALS. As Table 3 shows, when lymphocytes are stimulated with high levels of anti-HL-A any ¹²⁵I-antibody already associated with the nucleus is released.

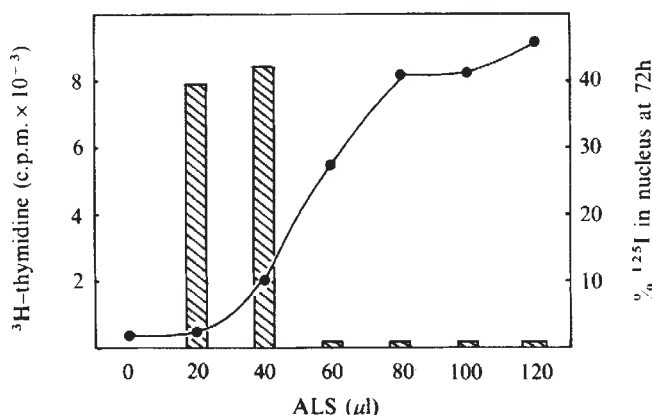


Fig. 4 DNA synthesis at 72 h in lymphocytes treated with various quantities of anti-lymphocyte serum (ALS). 10^6 cells ml^{-1} were maintained at 37° C in Eagles MEM + 10% foetal calf serum containing 0–120 $\mu\text{l ml}^{-1}$ ALS. DNA synthesis was assessed over a 16 h period at the end of culture by the addition of 2.5 $\mu\text{Ci ml}^{-1}$ ³H-thymidine. The amount of ¹²⁵I-ALS in the nucleus was determined as described in the legend to Fig. 3. 6×10^7 lymphocytes were treated for 30 min at 37° C with 0.1 ml of labelled serum. After washing the cells were distributed into vials containing medium with the various concentrations of ALS shown in the figure. Cell fractionation was carried out at 72 h.

Some idea of the mechanism which leads to the stimulation of lymphocytes in this experiment can be obtained by repeatedly treating cells with antiserum (Fig. 5). One day after the first sensitisation all complement dependent cytotoxicity was lost although ¹²⁵I-anti-HL-A2 was retained in the nucleus. After escaping from a primary challenge, cells could be resensitised by treating them a second time and a second escape cycle then took place. Parallel experiments using labelled antiserum showed that the second treatment initially serves to supplement the amount of label which remains after the first escape. When the second cycle of escape is complete, however, the total amount of labelled antibody in the cell is much greater than after the first cycle, and cell fractionation shows that the additional label is accumulated by the nucleus.

This amassing of antibody or a catabolised form of antibody in or around the nucleus therefore seems to be a step which is essential for lymphocyte triggering and although we do not yet know what processes are involved in this nuclear localisation it is thought that antibody is able to function as a derepressing agent at this site. With low levels of antiserum there is no

Table 3 Amounts of ^{125}I -labelled anti-HL-A8 in the various cell fractions during stimulation of cells with antiserum

	Initial	c.p.m.		
		24 h	48 h	72 h
Cells	1,749.2	735	756	53.1
Cytoplasm	1,839.8	10.6	10.8	29.7
Nucleus	14.9	699.0	761.1	47.6
DNA synthesis ^3H -thymidine c.p.m./ 10^5 cells	822.9	475.5	563.7	1,077.0

Cells were sensitised with ^{125}I -labelled antiserum for 30 min at 37°C , washed, and incubated in Eagle's MEM containing $70\text{ }\mu\text{l ml}^{-1}$ of cold anti-HL-A serum. DNA synthesis was estimated in separate cultures as described above.

stimulation of DNA synthesis and it is probable that lymphocyte mitotic activity is initiated only when the levels in the nucleus reach a certain threshold. Although it has been suggested that the patching and capping process which follows the treatment of cells with an antiserum is a prerequisite for lymphocyte triggering⁵, our results suggest that additional nuclear events are equally important.

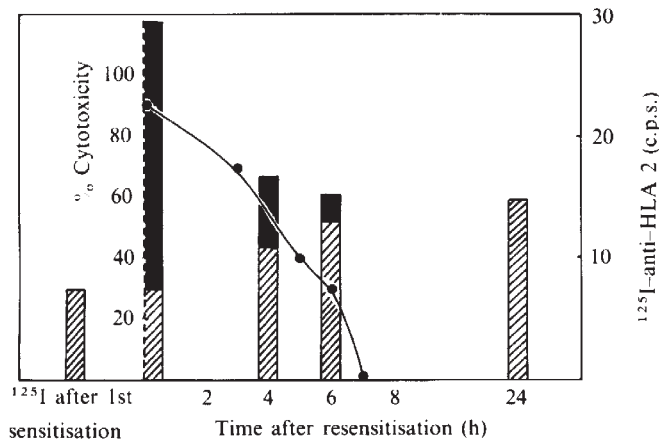


Fig. 5 Resensitisation of lymphocytes with an anti HL-A2 serum (the same quantity as for the first sensitisation). Complement-dependent cytotoxicity and cellular distribution of ^{125}I -anti HL-A were assessed; $\square$, amounts in the nucleus; $\blacksquare$, amounts in the remainder of the cell.

There is already some evidence that phytohaemagglutinin and other mitogens may be localised in the nucleus prior to lymphocyte stimulation¹⁴⁻¹⁶ and although it has been shown that mitotic activity can be initiated without the mitogen entering the cell this only happens when mitogens have been complexed in some way^{17,18}.

Our results show that the cell is capable of internalising anti-HL-A and antilymphocyte antibodies in much the same way as it internalises mitogens, and this seems to be necessary for this type of lymphocyte stimulation. We consider that the nucleus of a cell may play an equally important role in immunological events as changes which take place at the surface of the cell.

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Received October 25, 1973.

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Survival of Unmodified Spleen Allografts in Rats

LABORATORY models which provide for reproducible acceptance of organ allografts with neither preoperative conditioning or postoperative immunosuppression of recipient animals are rare. In pigs, however, liver allografts are generally accepted even if histocompatibility differences are strong¹⁻³; and indefinite acceptance of renal allografts in rats across major Ag-B barriers has been described⁴.

I have described the fate of spleen allografts between PVG and AGUS rats⁵. Grafts were rejected within 3 weeks but survived indefinitely when recipients were injected with cells from the donor strain before spleen grafting. Subsequent PVG skin grafts to spleen-bearing rats survived indefinitely when given 6 weeks or later after spleen grafting. Third party skin grafts were rejected as in controls. When long surviving spleen grafts were removed, recipients continued to accept further PVG skin allografts.

I hypothesised that the pretreatment had mitigated host-versus-graft (HvG) and supported a more enduring graft-versus-host (GvH) activity. I therefore felt that spleen allografts might survive without any recipient conditioning where donor-recipient reactivity was more balanced.

I describe here spleen allografts in inbred rats across strong Ag-B barriers without pretreatment or postoperative immunosuppression. Skin allograft survival was used to monitor recipient response towards donor and third-party tissue.

Three inbred rat strains were used: Wistar rats (Ag-B 1), AGUS rats (Ag-B 2), and PVG rats (Ag-B 5), obtained from Medical Research Council Laboratory Animals Centre, Carshalton, Surrey. Male or virgin female rats weighing approximately 200 g served as donors and recipients. In the recipient operation the spleen with an aortic cuff bearing the coeliac axis, and the portal vein were anastomosed end-to-side to the abdominal aorta and vena cava⁵. The recipient's own spleen was not removed. Full-thickness skin grafts with areas of 2×3 cm were sewn to graft beds on the back and covered for 7 d.

Criteria for spleen survival were both macroscopic and histological appearances at repeated laparotomies. The criterion for skin graft survival was hair growth. Rats dying within 2 weeks of operation through technical failure

were excluded from this report. None of the excluded rats showed signs of lethal GvH disease.

Table 1 Spleen allograft survival

Group	No. of animals	Donor	Recipient	Graft survival
1	22	PVG	AGUS	2-3 week
2	13	AGUS	PVG	7-8 month *
3	25	Wistar	AGUS	6-10 month *
4	11	AGUS	Wistar	3 month *

* Animals are still alive with viable spleen grafts.

Spleen grafts from PVG to AGUS rats were rejected within 3 weeks, whereas spleen grafts from AGUS to PVG, Wistar to AGUS and AGUS to Wistar rats are without exception still viable up to 10 months after grafting (Table 1). The grafts grew to between three to six times normal size. Maximum size was reached after about 3 weeks. Then instead of being rejected, as the spleens of group 1, the grafts remained viable with a good circulation and showed a tendency to reduce slowly in size. They were fairly soft in consistency, whereas rejecting grafts became swollen, hard, and patchy, then white and necrotic, and were finally absorbed completely. Histological findings of surviving spleens in groups 2 to 4, and the recipients' own spleens did not differ from those described recently⁵.

Table 2 Skin allografts to rats of Table 1 carrying spleen allografts

Group	No. of animals	Donor	Recipient	Time between spleen and skin grafting	Graft survival
1	8	PVG	AGUS	2 months	8.4 ± 0.5 d
2	7	AGUS	PVG	4 months	3 months *
3	7	Wistar	Agus	4 months	4-5 months *

* Animals are still alive with viable spleen and skin grafts. Animals of group 4 have not yet been skin grafted.

Skin grafts reflected the fate of the spleen grafts: skin grafts to rats of group 1, where the spleen grafts had been rejected, were rejected like controls, whilst skin grafts are still viable three to five months later after grafting on rats of groups 2 to 3 (Table 2). Spleen grafted rats rejected third-party skin allografts (Table 3). In all four groups, control skin allografts were rapidly rejected (Table 4).

It was demonstrated that the rat spleen allograft can be accepted and then induce a donor-specific tolerance to subsequent skin allografts. Some of the questions arising were: (1) were the recipients peculiar from an immunological point of view? (2) was the grafted organ lacking antigenicity? (3) to what extent was spleen graft survival dependent on immunological competence and competition of both graft and host?

Table 3 Third party skin allografts to rats of Table 1 carrying spleen allografts

Group	No. of animals	Donor	Recipient	Time between spleen and skin grafting	Graft survival
1	6	Wistar	AGUS	4 months	9.1 ± 0.4 d
2	7	Wistar	PVG	4 months	7.6 ± 0.8 d
3	6	PVG	AGUS	4 months	8.1 ± 0.3 d

In answer to question (1), all three rat strains behaved as if immunologically normal. They rapidly rejected control skin grafts. Heterotopic cardiac allografts exchanged between these rats were quickly rejected⁶. There were good responses in MLC between the strains used.

In answer to question (2), the spleen grafts were recognised as foreign by recipients in all combinations. In group 1 they were rejected. In groups 2 to 4 they survived initial HvG. Histological signs of early graft damage were apparent but

finally a state of equilibrium seemed to have been reached with maintenance of the circulation and viability of the tissue.

Table 4 Control skin allografts in strain combinations used for spleen grafting

Group	No. of animals	Donor	Recipient	Graft survival
1	9	PVG	AGUS	7.7 ± 0.3 d
2	7	AGUS	PVG	8.8 ± 0.6 d
3	6	Wistar	AGUS	9.4 ± 0.7 d
4	7	AGUS	Wistar	8.2 ± 0.4 d

In answer to question (3), a basic requirement for spleen graft survival seemed to be immunological competence and undisturbed competition of graft and host. Two results supported this: first, spleen allografts from AGUS rats to sublethally irradiated PVG recipients induced lethal GvH disease; second, spleen allografts from sublethally irradiated AGUS donors to PVG rats were promptly rejected (unpublished data). This implied that spleen allografts resulted in a confrontation of two immunocompetent systems. Their interaction might determine the rather puzzling outcome of graft survival. Interference with this interaction of graft and host was fatal for one side.

Follow-up skin grafts showed that rats with a viable spleen allograft were rendered specifically unresponsive towards the spleen donating strain, but not other strains. The spleen seemed to be a non-vital organ but it may prove to play a role in processes leading to allograft acceptance.

Immunosuppression in clinical transplantation is entirely aimed at inhibiting host action against the graft. But it prevents, as a side effect, graft-versus-host action as well. More knowledge about the mechanisms leading to the results described might initiate a second look at current treatment after transplantation of organs. It might be possible to encourage, instead of inhibiting graft and host reactivities, having in mind their balanced immunological interaction and not paralysis.

I thank Dr B. M. Herbertson, Department of Pathology, Cambridge, who did all the histological examinations, and Professor R. Y. Calne, Department of Surgery, Cambridge for guidance and advice.

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Received October 16; revised November 23, 1973.

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Sensitivity to Amphotericin B of *in vitro* Established Cell Lines

It has been demonstrated that the antifungal lipophilic polyene amphotericin B inhibits eukaryotic cell growth by binding to sterols and making 'holes' in cell membranes¹. Used at subinhibitory concentrations, it enhances penetration of other antibiotics into the cells of higher organisms *in vitro* and *in vivo*, showing a synergistic effect with a number of unrelated drugs^{1,2}. It has also been demonstrated that higher doses of amphotericin B inhibit macromolecular syntheses, probably by severely damaging the cell membrane³.

Table 1 Cell lines used

Cell line	Origin	Characteristics	Reference
3T3	Mouse		10
3T3-TK ⁻	Mouse	3T3, thymidine kinase deficient	11
3TP	Mouse	3T3-TK ⁻ , puromycin resistant	12
3T3-SV	Mouse	3T3, transformed by SV40	13
BHK-B1	Syrian hamster	BHK, thymidine kinase deficient	14
BHK-T6	Syrian hamster	BHK, hypoxanthine guanine phospho-ribotransferase (HGPRT) deficient	15
WG3	Chinese hamster	DON, HGPRT deficient	16
K455	Human	HGPRT, deficient, primary explant (Lesh-Nyhan)	Personal communication (from Klinger through Siniscalco)

We report here our investigations on the sensitivity to amphotericin B of several *in vitro* established cell lines, and of hybrid clones derived from two lines which exhibited very different degrees of sensitivity. We observed a positive correlation between levels of density-dependent inhibition of cell growth and sensitivity to the drug.

A number of cell lines (Table 1) was tested for sensitivity to amphotericin B according to the method described by Medoff *et al.*⁴. This is based on the incorporation of ³H-uridine into acid-precipitable material during an 18 h incubation of actively growing cells in Dulbecco's modified Eagle medium (GIBCO), supplemented with 10% foetal calf serum (Gray) and in the presence of increasing concentrations of the drug (Fungizone GIBCO). Figure 1 shows that some cell lines were extremely sensitive to the drug (3T3, 3T3-TK⁻, K455), some were highly resistant (3T3-SV, BHK-T6, WG3), while others had an intermediate sensitivity (3TP, BHK-B1). When ordering these lines with regard to their sensitivity to amphotericin B (Table 2), we observed that the more sensitive ones were those showing a more severe contact inhibition of cell growth, as evaluated by the correlation with their efficiency of plating (EOP) in agar⁵ and saturation density⁶, despite the high variability in cell size of the various lines used. The finding that SV40-transformed 3T3 cells showed a very high degree of resistance was consistent with this observation.

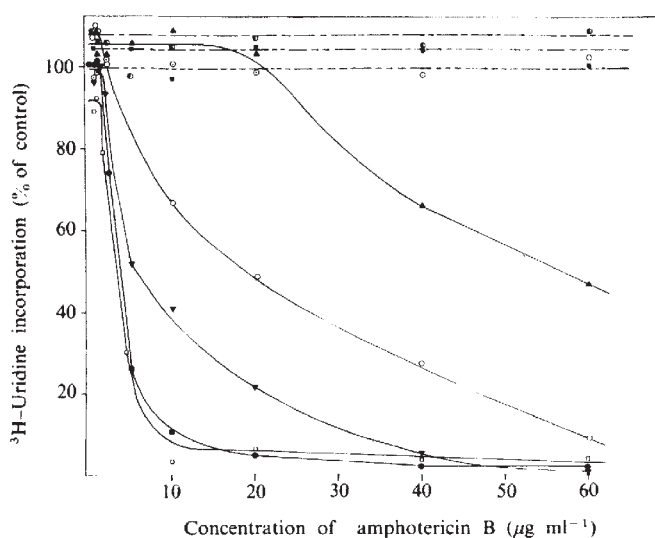


Fig. 1 Effect of amphotericin B on the incorporation of ³H-uridine into TCA-precipitable material of various cell lines. ○—○, BHK-T6; ■—■, 3T3-SV; ○—○, WG3; ▲—▲, BHK-B1; ○—○, 3TP; ▼—▼, 3T3; ●—●, 3T3-TK⁻; □—□, K455.

To better analyse the observed correlation, we selected from 3T3-TK⁻ cells (a highly contact-inhibited line) three clones capable of growing in agar by forcing growth beyond confluence for several passages. These three clones were tested

Table 2 Amphotericin B sensitivity, saturation density and efficiency of plating in agar of cell lines used

Inhibitory concentration of amphotericin B* (µg ml ⁻¹)	³ H-uridine incorporation at 60 µg ml ⁻¹ of amphotericin B (% of control)	Cell line	Saturation density (× 10 ⁴ cells cm ⁻²)	EOP in agar
2-5	2	3T3-TK ⁻	4.2	< 10 ⁻⁵
2-5	4	K455	4	< 10 ⁻⁵
5-10	1	3T3	4.5	< 10 ⁻⁵
10-20	9	3TP	30	ND
40-60	48	BHK-B1	48	3 × 10 ⁻⁴
> 60	100	WG3	60	6 × 10 ⁻⁴
> 60	100	3T3-SV	35	7 × 10 ⁻²
> 60	100	BHK-T6	61	4 × 10 ⁻⁴

* Concentration of amphotericin B which inhibits the incorporation of ³H-uridine to 50% of control.

for their sensitivity to amphotericin B (Fig. 2), and all showed a significantly increased resistance, as compared with the parental line. In addition, all the clones showed higher saturation densities than the parental line (Table 3).

Table 3 Characteristics of three sublines of 3T3-TK⁻ selected in agar medium

Subclone	Inhibitory concentration of amphotericin B* (µg ml ⁻¹)	³ H-uridine incorporation at 60 µg ml ⁻¹ of amphotericin B (% of control)	Saturation density (× 10 ⁴ cells cm ⁻²)
KW-1	~ 10	32	19
KW-2	~ 10	34	18
KW-4	~ 10	28	21
3T3-TK ⁻	2-5	2	4.2

* Measured as in Table 2.

When a rare survivor from 3T3-TK⁻ cells treated with 20 µg ml⁻¹ of amphotericin B was tested for its ability to grow in agar, or its saturation density was measured, we again observed a correlation between resistance to the drug and release from contact inhibition of growth measured as saturation density (25 × 10⁴ cells cm⁻²).

To study the behaviour of amphotericin B sensitivity in cell hybrids between lines with extreme degrees of sensitivity, we selected spontaneous cell hybrids from BHK-T6 and 3T3-TK⁻ cells by Littlefield's method⁷ with techniques already described⁸.

Twelve hybrid clones were selected and tested for their sensitivity to amphotericin B, saturation density and chromosomal constitution. Results are shown in Table 4. Judging by the shape of inhibition curves shown in Fig. 3, characterised by an initial strong decrease of ³H-uridine incorporation similar to that of the 3T3-TK⁻ parent and after an intermediate sensitivity between the two parental lines, hybrid lines may be composed of mixtures of cells exhibiting essentially two different levels of sensitivity to the drug.

Since mouse chromosomes are easily lost from hamster-mouse hybrids⁹, it seemed possible that the observed levels of

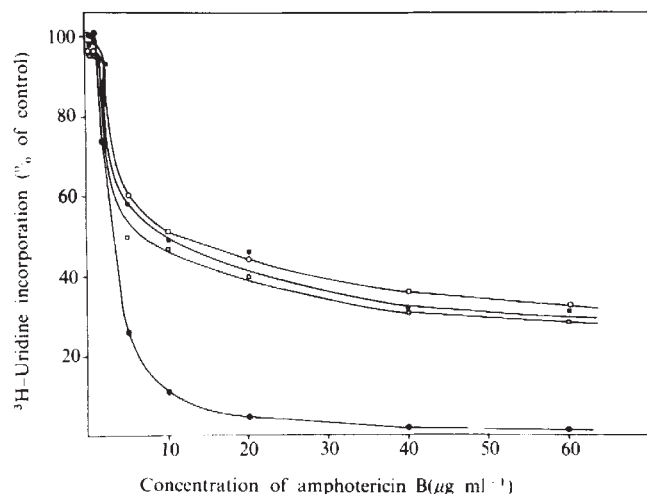


Fig. 2 Effect of amphotericin B on the incorporation of ^3H -uridine into TCA-precipitable material of clones derived from 3T3-TK $^-$ cells. $\circ$ — $\circ$, KW1; $\square$ — $\square$, KW2; $\blacksquare$ — $\blacksquare$, KW4; $\bullet$ — $\bullet$, 3T3-TK $^-$.

sensitivity to amphotericin B were correlated with different numbers of mouse chromosomes present in the hybrids. To test this possibility the hybrid line 21 exhibiting intermediate sensitivity was further subcultured in hypoxanthine-aminopterin-thymidine (HAT) medium for approximately one hundred generations. These cells were then resistant to high levels of amphotericin B (Fig. 3), their saturation density had increased to 35×10^4 cells cm^{-2} , and their chromosomal constitution had changed significantly (telocentric/total = 0.30).

The hybrid cell lines analysed exhibited an increased sensitivity to amphotericin B compared to hamster parent proportional to the ratio of the mouse chromosomes they carry

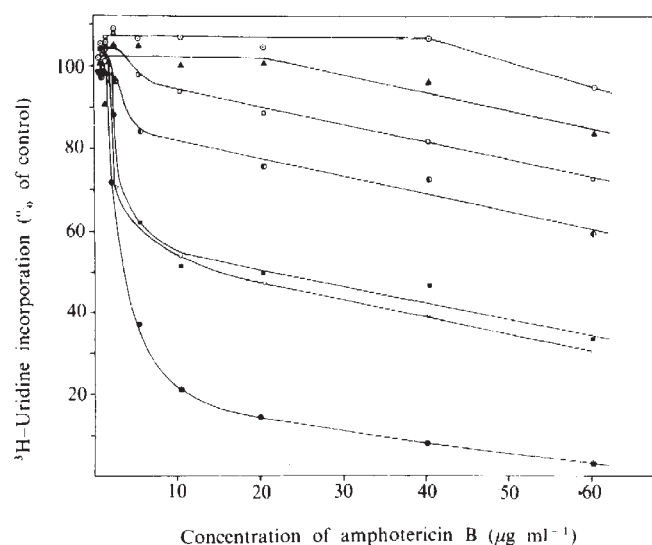


Fig. 3 Effect of amphotericin B on the incorporation of ^3H -uridine into TCA-precipitable material of some representative hybrid lines derived by fusion of BHK-T6 $\times$ 3T3-TK $^-$ cells. $\bullet$ — $\bullet$, Hy 23; ∇ — ∇ , Hy 1; $\blacksquare$ — $\blacksquare$, Hy 21; $\circ$ — $\circ$, Hy 8; $\square$ — $\square$, Hy 17; $\blacktriangle$ — $\blacktriangle$, Hy 32; $\circ$ — $\circ$, Hy 21, after a hundred generations from first determination.

Table 4 Levels of sensitivity to amphotericin B, saturation density and chromosomal constitution of BHK-T6 $\times$ 3T3-TK $^-$ hybrid lines

Hybrid line	Inhibitory concentration of amphotericin B* ($\mu\text{g ml}^{-1}$)	^3H -uridine incorporation at $60 \mu\text{g ml}^{-1}$ of amphotericin B (% of control)	Saturation density ($\times 10^4$ cells cm^{-2})	Telocentric $\dagger$ total
1	10–20	30	10	0.48
4	> 60	87	41	0.28
8	> 60	60	22	0.35
13	10–20	32	13	0.46
17	> 60	72	20	0.36
21	10–20	34	10	0.58
22	10–20	27	9	0.48
23	2–5	3	3.5	0.62
26	10–20	29	11	0.46
32	> 60	84	37	0.28
35	> 60	82	40	0.30
39	10–20	32	12	0.45

* Measured as in Table 2. See Fig. 4.

$\dagger$ Mean of at least ten karyotypes done after 3 week from hybridisation and at the time of amphotericin B sensitivity and saturation density determination. Telocentric chromosomes are mainly of mouse origin 9 .

(Table 4), thus showing dominance of the mouse sensitivity character over the hamster resistant one.

The drug sensitivities of the hybrid lines could be accounted for if the individual lines were mixed, containing various relative fractions of sensitive cells still carrying the relevant mouse chromosome and cells that have lost the chromosome and become resistant. It could also be that the lines are individually more or less pure and the drug inhibition pattern is gene dose dependent and characteristic of the multiplicity of the relevant mouse chromosomes present in the line. The segregation of resistant cells after further cultivation in HAT medium substantiates the proposal that the sensitivity derives from the presence of mouse chromosomes in the hybrid cell lines.

Since the continuous selection in HAT medium did not interfere with the segregation of hybrid cells resistant to amphotericin B, as in the case of hybrid line 21, it seems that the mouse allele responsible for sensitivity to the drug is not linked to the HGPRT allele.

Cells with reduced membrane-mediated control of growth (reduced levels of contact inhibition) exhibit increased levels of resistance to amphotericin B. Cells exhibiting increased levels of resistance to amphotericin B show reduced levels of contact inhibition. We conclude the action of amphotericin B on cell membranes is mediated through a membrane component involved in regulation of growth.

It is also apparent that amphotericin B, which is commonly used as an antifungal agent in cell culture media, imposes selection in favour of cells with decreased membrane-mediated growth control. We are examining the possibility that the use of amphotericin B may provide a convenient selective agent for virus-transformed cells.

We are grateful to Professor D. Schlessinger for suggesting the use of amphotericin B for its synergistic property, for informing us of his work before publication, and together with Professor M. Fox, for reviewing the manuscript. We thank Professor G. Marin for discussions and Mrs M. Terracciano for technical help.

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Received September 25; revised November 14, 1973.

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Increased Mobility and Redistribution of Concanavalin A Receptors on Cells infected with Newcastle Disease Virus

CONCAVALIN A (con A) and various other plant lectins have been used extensively to study differences in the organisation of the surface of normal and transformed cells¹⁻³. Cells transformed by oncogenic viruses are agglutinated by doses of con A which do not affect normal cells, but the latter can be made susceptible to agglutination by small doses of con A by brief trypsinisation. Furthermore, the susceptibility of normal cells to agglutination by con A can be increased by infection with various non-oncogenic enveloped viruses, including herpesviruses^{4,5}, poxviruses⁶, arboviruses⁷, paramyxoviruses^{8,9} and rhabdoviruses⁹. The mechanism of this increased agglutinability is unknown. Since the glycoproteins in the envelope of such viruses can bind con A⁹⁻¹¹, their insertion into the plasma membrane of the infected cell during virus envelopment and release might increase the available con A receptors, which could account for the increased susceptibility to agglutination. We have detected no significant increase in con A receptors on cells infected with Newcastle disease virus (NDV) which were susceptible to agglutination by con A at concentrations that did not affect normal uninfected cells. We have found, however, that the increased agglutinability is due to a redistribution of con A receptors on the cell surface.

Baby hamster kidney (BHK) cells were cultured in Dulbecco's modified Eagle's medium in 60 mm plastic Petri dishes (Falcon) as described previously¹². The origin and properties of the NDV strains¹³⁻¹⁵, methods for infection of cell cultures^{14,15} and measurement of the susceptibility of cells to agglutination by con A⁴ have also been described. Con A was obtained as a twice crystallised protein (Miles-Yeda) and purified further by affinity chromatography¹⁶. Labelled con A was prepared from highly purified con A using ³H-acetic anhydride¹⁷. ³H-con A (specific activity 3×10^6 c.p.m. per mg con A) resembled native con A in its reactivity with rabbit anti-con A antiserum, co-chromatographed with unlabelled con A on DEAE-cellulose and agglutinated cells in doses similar to native con A. Binding of ³H-con A to cells was measured at 0° C, 21° C and 37° C as described previously¹⁸. To calculate the specific binding of ³H-con A, the amount bound in the presence of 0.1 M α -methylmannoside was subtracted from the amount bound in its absence. Kinetic analysis of ³H-con A binding revealed saturation binding after 30 min of incubation at 0° C, 21° C and 37° C.

The distribution of receptors on the cell surface was assessed by immunofluorescence using fluorescein-conjugated anti-con A antibodies¹⁹⁻²¹, induced in rabbits by four subcutaneous injections of 2 mg purified con A in Freund's complete adjuvant followed by two intraperitoneal inoculations of 2 mg con A in alum. Rabbits were bled 1 week after the last injection, the anti-con A globulin-containing fraction was

isolated from the serum and conjugated with fluorescein isothiocyanate^{21,22}. Cells were exposed to con A at 0° C, 21° C and 37° C followed by further incubation at the appropriate temperature and then labelled with the fluorescein-conjugated anti-con A antibodies (F-anti-con A) for 1 h at the same temperature. Finally, the cells were fixed with 2.5% glutaraldehyde and the specific immunofluorescence pattern examined in a Leitz Ortholux microscope using ultraviolet illumination with a UG-1 excitation and K-430 barrier filter. As outlined below, in some experiments the cells were prefixed with glutaraldehyde before adding con A.

Measurements of ³H-con A binding at 21° C or 37° C to BHK cells infected with virulent (Herts; Texas) mesogenic (Beaudette C) and avirulent (Queensland) strains of NDV revealed no significant difference from mock-infected cells (Fig. 1). Noonan and Burger²³ reported that measurements of con A binding made at room temperature or 37° C give misleading results and do not detect any significant differences in binding between cells. They proposed that true differences in con A binding to cell surfaces are obscured at these temperatures due to non-specific binding and endocytosis of bound con A. They reported that these problems were avoided if con A binding was measured at 0° C. However, measurement of ³H-con A binding at 0° C to NDV-infected and uninfected BHK cells revealed that significantly less lectin was bound to cells than at higher temperatures but there was no significant difference in binding between infected and uninfected cells (Fig. 1).

Thus the increased agglutinability of NDV-infected cells does not seem to be due to an increase in available con A receptors; nor to changes in the size of cells after infection¹⁸.

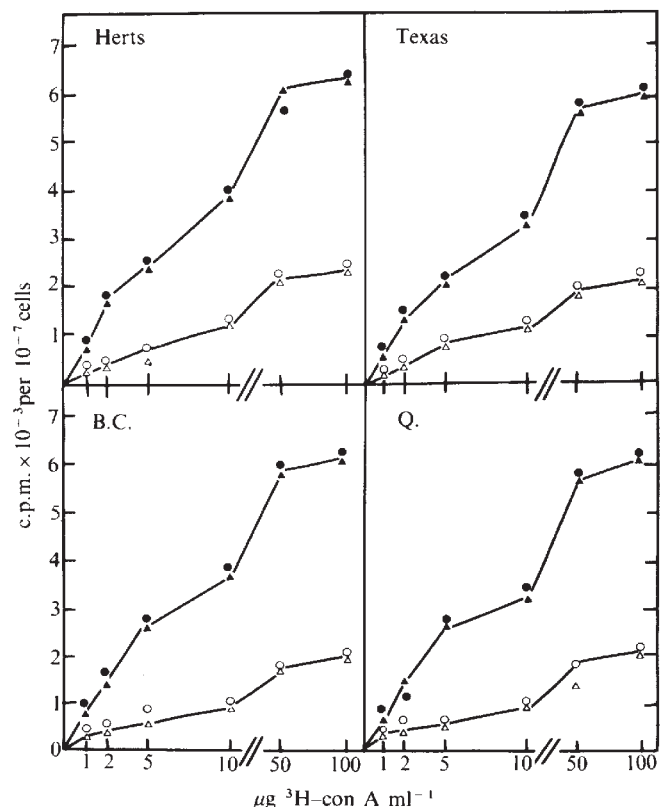


Fig. 1 Specific binding of ³H-con A at 21° C and 37° C (closed symbols) or 0° C (open symbols) to BHK cells and BHK cells infected with NDV strains Herts, Texas, Beaudette C (B.C.) and Queensland (Q.). ● and ○, infected cells; ▲ and △, uninfected cells. The results for binding of ³H-con A at 21° C and 37° C were identical and for convenience are shown together. Binding measurements were made 7, 8 and 10 h after infection with strains Herts, Texas and B.C., respectively, at which times the cells were susceptible to agglutination by 500 μ g con A ml⁻¹ which failed to agglutinate uninfected control cells. In cells infected with strain Queensland binding was measured 16 h after infection.

It is possible, however, that the failure to detect an increase in con A receptors on NDV-infected cells, in spite of insertion of con A-binding viral proteins into the plasma membrane, is due to steric hindrance between adjacent con A molecules. For example, the con A receptors, including any new sites created by viral proteins, might be able to pack more closely than can the con A molecules themselves. This problem applies equally, however, to the numerous reports that significant differences in con A binding were not detected between cells that agglutinated and those that did not¹⁻³.

Our failure to detect an increase of receptors on NDV-infected cells agglutinated by con A resembles findings with virus-transformed and protease-treated cells¹⁻³. With one exception²³, many studies have shown that the agglutination

of these cell types by small doses of con A is not due to an increase in con A receptors, but to their redistribution from a random pattern to form clusters^{2,21,24,25}. Differences in the agglutinability of cells are presumed to result from differences in the organisation of the cell periphery which affect the ease with which receptors can move within the membrane to form clusters. We have investigated whether the increased agglutinability of BHK cells infected with certain NDV strains involved a similar redistribution of con A receptors.

We examined the immunofluorescence staining pattern on cells treated with con A and F-anti-con A before fixation to provide information on the inherent pattern of the receptors at a given temperature. Similarly, cells exposed to con A at 4° C and fixed without warming before addition of F-anti-con A provide information on the inherent topography of the receptor sites since their movement is reduced at low temperatures^{21,24-26}.

Uninfected cells treated with con A and F-anti-con A before fixation developed a uniform 'ring' immunofluorescence pattern with fluorescence confined to the periphery of the cell (Fig. 2a), characteristic of a random distribution of con A receptors¹⁹⁻²¹. 'Patchy' fluorescence staining, characteristic of aggregated or clustered con A receptors¹⁹⁻²¹, was observed on less than 3% of cells at 4° C and 37° C (Fig. 2a). As a control to test the accuracy of the staining method, normal cells were also trypsinised to induce redistribution of con A receptors into clusters¹⁹⁻²¹. These cells, treated with con A at 4° C, showed predominantly the same uniform ring fluorescence pattern (Fig. 2b). Most trypsinised cells treated with con A at 37° C, or at 4° C followed by warming to 37° C, however, showed patchy fluorescence. The trypsinised cells also showed a maximum agglutination response to 500 µg con A ml⁻¹ while non-trypsinised cells were unaffected.

Observations on the distribution of con A receptors on BHK cells infected with the Beaudette C strain of NDV, and which showed increased agglutinability, revealed uniform fluorescence on cells exposed to con A at 4° C, but most of the cells exposed at 37° C showed patchy fluorescence indicative of clustering of the receptors (Fig. 2c). Similarly, infected cells treated with con A at 4° C and warmed to 37° C before fixation showed predominantly patchy fluorescence (Fig. 2c). The change from predominantly uniform to predominantly patchy fluorescence after infection with Beaudette C occurred as the cells became increasingly susceptible to agglutination (Fig. 3a). Most cells infected with the avirulent NDV strain Queensland, which show no increased susceptibility to agglutination by con A compared with uninfected cells, revealed a consistent uniform fluorescence pattern throughout infection (Fig. 3b).

In contrast to the 'patchy' fluorescence found on most Beaudette C-infected cells treated with con A before fixation, staining of similar infected cells fixed with glutaraldehyde before addition of con A revealed uniform fluorescence (Fig. 2d). This indicates that the inherent topography of con A receptors on infected cells which show high agglutinability is no different from that on uninfected cells which fail to agglutinate. This suggests that the increased agglutinability of infected cells is not caused directly by virus-induced changes in the topography of con A receptors on the cell surface. Instead, the clustering of receptors is probably due to their greater mobility within the membrane, and is probably a secondary phenomenon induced by exposure to con A, presumably as a result of the cross-linking of adjacent receptors by tetravalent con A molecules. The mechanism by which infection with certain NDV strains could alter the cell surface to permit greater mobility of the con A receptors within the membrane is unknown.

These results reinforce the overall similarities between con A agglutination of normal cells infected with non-oncogenic viruses and the agglutination of transformed and protease-treated cells by similar doses of con A. For example, three studies have shown that neither transformation nor proteolytic modification of the cell surface lead directly to clustering of

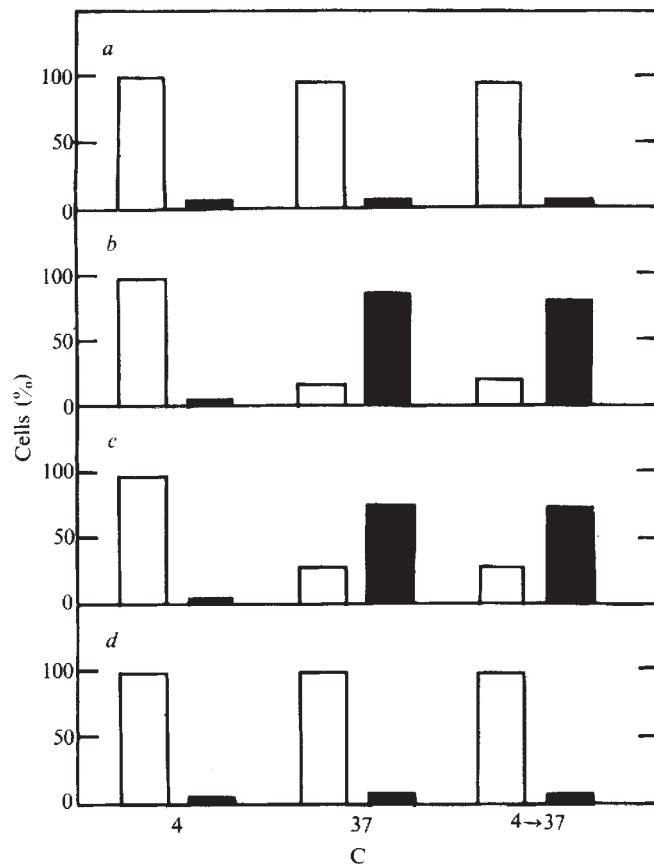


Fig. 2 The distribution of con A receptors on BHK cells detected by immunofluorescence using fluorescein-conjugated rabbit anti-con A antibodies (F-anti-con A) as described in the methods. Cells were examined after exposure to con A and F-anti-con A at 4° C, 37° C or exposure to con A at 4° C followed by warming and incubation at 37° C for 30 min before addition of F-anti-con A (4→37° C). The results are expressed as the percentage of cells showing uniform ring fluorescence (□) characteristic of randomly dispersed con A receptors or showing 'patchy' fluorescence (■) characteristic of clustered con A receptors. The results are derived from three replicate experiments and a minimum of at least fifty cells were examined in each experiment. *a*, Receptor distribution at different temperatures on uninfected BHK cells exposed to con A and F-anti-con A before final fixation. *b*, Receptor distribution at different temperatures on BHK cells 1 h after treatment with 0.01% twice crystallised trypsin for 10 min at 37° C. The cells were treated with con A and F-anti-con A before final fixation. *c*, Receptor distribution at different temperatures on BHK cells 10 h after infection with NDV strain Beaudette C and exposed to con A and F-anti-con A before final fixation. The cells were susceptible to agglutination by 500 µg con A ml⁻¹ at 21° C or 37° C but uninfected cells were unaffected; *d*, receptor distribution at different temperatures on BHK cells 10 h after infection with NDV strain Beaudette C and fixed with 2.5% glutaraldehyde for 15 min at the appropriate temperature before exposure to con A and F-anti-con A.

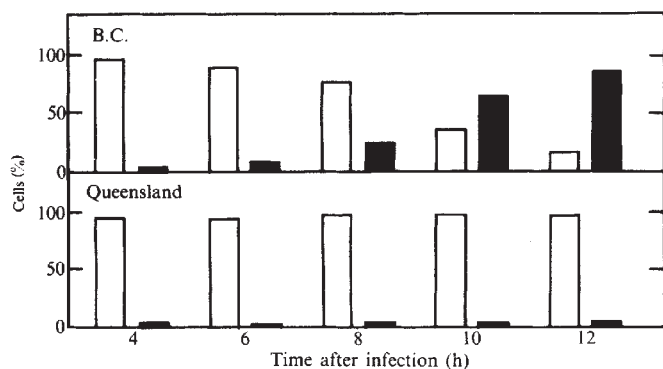


Fig. 3 The distribution of con A receptors on BHK cells at intervals after infection with NDV strains Beaudette C (B.C.) and Queensland. Immunofluorescence measurements were made on cells exposed to con A and F-anti-con A at 37°C before final fixation and the results determined as in Fig. 2. □, Uniform fluorescence and ■, patchy fluorescence. Cells infected with strain B.C. showed an increased susceptibility to agglutination by 500 µg con A ml⁻¹ from 8 h after infection (+) and by 12 h after infection more than 90% of the cell population were agglutinable (+++). Cells infected with strain Queensland were not susceptible to agglutination by this dose of con A for up to 48 h after infection.

con A receptors^{21,24,25}. These, and our new findings, indicate that the inherent topography of con A receptors on cells with a high susceptibility to con A agglutination does not differ from that of normal cells which are not agglutinated, and that receptors cluster only after exposure to con A at room temperature or above. Thus, it seems that a non-oncogenic virus such as NDV, malignant transformation by viruses and protease-treatment can all induce greater mobility of con A receptors within the plasma membrane. Whether or not a common mechanism is operating in each situation remains to be resolved.

This work was supported by grants from the National Cancer Institute and the Agricultural Research Council of Great Britain. We thank P. Newhouse, F. Dutton and A. MacKearnin for technical assistance.

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Effects of Cytochalasin B on Uptake and Release of Putative Transmitters by Synaptosomes and on Brain Actomyosin-like Protein

A LARGE variety of contractile cellular functions is thought to be associated with microfilamentous processes which can be disrupted by the fungal metabolite cytochalasin B (ref. 1). To this list of contractile processes have been added the stimulated release of secretory products²⁻⁴ as well as transmitter agents⁵. Based in part on the isolation of actomyosin-like protein (neurostenin) from mammalian brain nerve ending preparations⁶ a mechanism has been suggested for the exocytotic release of transmitter materials at synaptic endings involving contractile events similar to those occurring during muscle contraction⁷. We have examined the effect of cytochalasin B on the uptake by rat whole brain nerve ending preparations of noradrenaline, dopamine, glutamic acid and GABA and their K⁺-stimulated release. We also studied the effect of cytochalasin B on the adenosine triphosphatase activity of the brain actomyosin-like protein and muscle actomyosin.

The assay system for the uptake and release studies was the same as that used previously⁸. The cytochalasin B (ICI) was prepared as a 0.1 M solution in dimethylsulphoxide (DMSO) and added to a final concentration of 0.15 mM. Control studies were run with the same amounts of DMSO. In one set of experiments the synaptosome preparation was incubated with cytochalasin B for 15 min before the addition of 7-³H-L-noradrenaline, 1,2-³H-dopamine, U-¹⁴C-L-glutamic acid or 1-¹⁴C-4-aminobutyric acid (GABA) followed in 15 min (5 min for glutamic acid) by the addition of 1 M KCl to a final concentration of 30 mM K⁺ (Fig. 1). The concentration of cytochalasin B used in these experiments was several-fold greater than that used by some workers⁴ but comparable to that used by others^{5,9}.

Cytochalasin B had a small but significant effect on the uptake of noradrenaline and dopamine (Fig. 1a). Its major effect was on the K⁺-stimulated release of noradrenaline (Fig. 1b); that on dopamine was less but also significant. Neither the uptake nor the release of glutamic acid or GABA was affected (Fig. 1, Table 1). Although the uptake of noradrenaline was inhibited approximately 20%, its release was inhibited by approximately 60% (Table 1). The effect on dopamine was not as dramatic; its uptake and release were inhibited approximately 30% and 25%, respectively (Table 1). Since there was no effect on uptake of amino acids, cytochalasin B seems not to influence energy metabolism in these experiments⁸.

In a second set of experiments, the synaptosome preparation was incubated with the radioactive tracer for 15 min before the addition of the cytochalasin B, followed in 15 min by KCl to a final concentration of 30 mM K⁺.

The noradrenaline, dopamine and GABA continued to

Table 1 Comparative Effects of Cytochalasin B on Uptake and K⁺-stimulated Release of Putative Transmitters

	% Control uptake*	% Control*	Release†
³ H-Noradrenaline	81 ± 5‡ (5)	38 ± 6‡ (5)	11 ± 4‡ (4)
³ H-Dopamine	69 ± 2‡ (5)	78 ± 6‡ (5)	68 ± 5‡ (4)
¹⁴ C-GABA	101 ± 4 (5)	98 ± 3 (5)	91 ± 8 (2)
¹⁴ C-Glutamate	103 ± 13 (4)	93 ± 1 (4)	—

* Derived from studies described in Fig. 1a and b.

† Derived from the experiments described in Fig. 1c and d.

‡ $P < 0.01$.

See Fig. 1 for experimental details. Uptake is defined as the ratio of: (d.p.m. mg⁻¹ protein on filter)/(d.p.m. total filtrate) and release is defined as the ratio of uptake after addition of 30 mM K⁺ to the uptake before that addition. The values are expressed as the percentage of uptake or release in the absence of cytochalasin B ± s.e. (*n*) is number of different preparations. Duplicate determinations were run with each preparation.

concentrate slightly in the tissue preparation during the second 15 min period even in the presence of the cytochalasin B (compare Fig. 1a with 1c). Cytochalasin B seemed to have no significant effect on the uptake of the compounds during this second 15 min period. In contrast, there was again a marked inhibition of the K⁺-stimulated release of noradrenaline (89%) (Fig. 1d); the effect on dopamine release was much less (30%) although also significant. There was no effect on the uptake or release of GABA.

The release of dopamine was much less affected than that of noradrenaline. The explanation for this may be that synaptosomes prepared from whole brains are more enriched with noradrenaline-specific endings than with dopamine-specific endings. The finding that neither the uptake nor release of glutamic acid and GABA were affected by cytochalasin B suggests that these substances are taken up and stored differently than the catecholamines^{8,10-12}. Noradrenaline and dopamine are presumably stored in the presynaptic vesicles of the nerve endings and released from these sites. If this is true, then the amino acids may not be similarly stored.

The actomyosin-like protein, neurostenin, was isolated from the synaptosomal fraction of rat brain⁶. The Ca²⁺ or Mg²⁺ stimulated adenosine triphosphatase activity was measured by the P_i released from ATP¹³. Incubation of the protein with cytochalasin B for 10 min at 37° C before the addition of substrate ATP (MgATP) resulted in approximately 50% inhibition of the Mg²⁺-stimulated adenosine triphosphatase activity with a concentration of 0.1 mM cytochalasin B (Fig. 2). Only slightly greater inhibition was achieved at 0.5 mM cytochalasin B. The Ca²⁺-stimulated adenosine triphosphatase activity was affected only to a small extent even at 0.5 mM cytochalasin B. With concentrations at or below 0.1 mM the inhibition of the reciprocal of the rate of ATP hydrolysis was proportional to the concentration of the cytochalasin B (Fig. 2). These data suggest the inhibition is of a non-competitive nature.

Table 2 Effect of 0.1 mM Cytochalasin B on Mg²⁺-stimulated Adenosine Triphosphatase Activity

Myosin + actin	Myosin + neurin % Control activity	Stenin + actin	Stenin + neurin
54 ± 5 (2)	31 ± 4 (4)	47, 15 (2)	56 ± 4 (4)

See Fig. 2 for details of assay conditions. The protein concentrations in the incubation mixtures were 0.01 to 0.02 mg ml⁻¹ for myosin and stenin and 0.02 to 0.05 mg ml⁻¹ for actin or neurin (*n*)=number of preparations. Duplicate determinations were made on each preparation.

The neurin and stenin were obtained after osmotic shock disruption of synaptosomal preparations⁷. The neurin was isolated either by direct extraction from the membrane fraction⁷

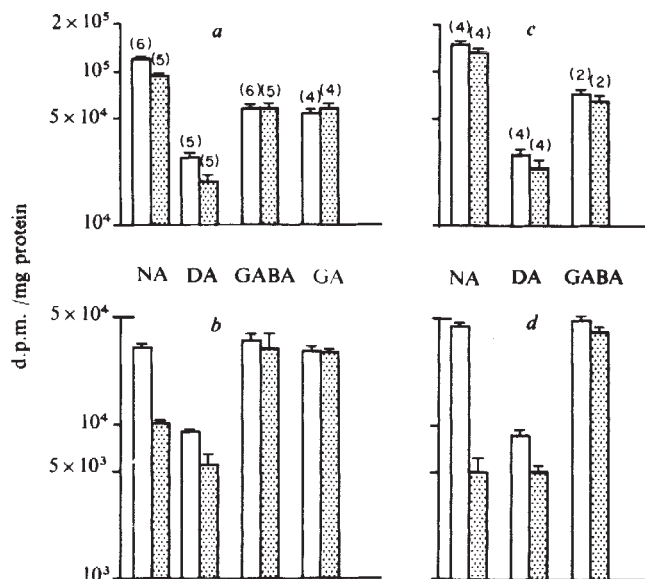


Fig. 1 Effect of cytochalasin B (CB) on the uptake by rat brain synaptosomes of noradrenaline (NA), dopamine (DA), 4-aminobutyric acid (GABA) and glutamic acid (GA) and their K⁺-stimulated release. □, Control; ■, 0.15 mM CB. *a, b*, Portions of crude synaptosomal preparations were incubated 15 min at 37° C with or without 0.15 mM cytochalasin B in a Krebs-Ringer phosphate buffer (pH 7.3–7.4) fortified with 10 mM glucose, 0.45 mM ATP and 10 mM pyruvate⁸. When NA or DA were used, the medium also contained 0.03% (w/v) ascorbate and 0.001% pargyline. When GABA or glutamate were used, 1 mM aminooxyacetic acid was added to the basic medium. The protein concentration was 1.0–1.5 mg ml⁻¹. At the end of the 15 min incubation period, L-³H-noradrenaline (6.6 Ci mmol⁻¹), 1,2-³H-dopamine (370 mCi mmol⁻¹), 1-¹⁴C-4-aminobutyric acid (3 mCi mmol⁻¹) or L-U-¹⁴C-glutamate (270 mCi mmol⁻¹) were added; the final concentrations were 0.20 μM, 0.25 μM, 20 μM and 0.2 μM, respectively. Fifteen minutes following addition of radioactivity (5 min for glutamate) 1 ml samples were removed from the incubation mixture and passed through a 0.65 μm Millipore filter. The filter was washed three times with 1 ml of 0.15 M NaCl. At the same time 1 M KCl was added to the incubation mixture to yield a final concentration of 30 mM K⁺. After 3 min, a 1 ml sample was removed for analysis and treated as above. Radioactivity on the filters and in the filtrates was measured with a Packard Tri-Carb liquid scintillation spectrometer. *a*, Radioactivity on the filter before addition of the KCl; *b*, radioactivity released into the medium by the addition of KCl. *c, d*, These experiments were similar to *a* and *b* except that the preparations were incubated with label for 15 min at 37° C and then 0.15 mM cytochalasin B added. The mixtures were then incubated for a further 15 min. At that time, a 1 ml sample was withdrawn for analysis and KCl added as before. *c*, Radioactivity on the filter before KCl addition; *d*, radioactivity released into the medium by addition of KCl. Since the cytochalasin B was added as a solution in dimethylsulphoxide, the controls received similar volumes of dimethylsulphoxide alone. (*n*)=number of separate preparations tested in duplicate. In *a, b* and *d*, the difference in d.p.m. mg⁻¹ protein between control and addition of cytochalasin B for NA and DA was significant at the $P < 0.01$ level by Student's *t* test.

or from the osmotic shock supernatant medium remaining after separation of the vesicle and membrane fractions. In the latter case, the supernatant was made 0.1 M in KCl, 1 mM in MgCl₂ and 0.5 mM in ATP. After 1 h at room temperature in the dark, the protein was allowed to polymerise overnight at 4° C. After centrifugation at 100,000g for 3 h the pellet was solubilised in 0.2 mM ascorbate, 0.2 mM ATP, pH 7.8 and the solution clarified by centrifugation for 20 min at 47,000g. The neurin prepared by the two procedures behaved similarly in the recombination studies described below and therefore the resulting data were pooled. The stenin was isolated from the osmotic shock vesicle fraction⁷. Actin and myosin were prepared from cat striated muscle^{14,15}.

In recombination studies (Table 2), 0.1 mM cytochalasin

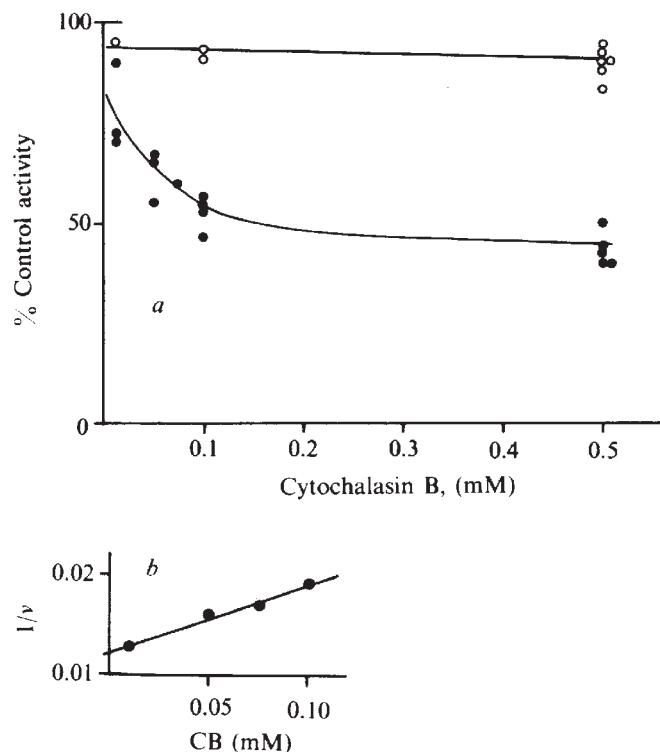


Fig. 2 *a*, Effect of cytochalasin B on the adenosine triphosphatase activity of actomyosin-like protein (neurostenin) isolated from rat brain synaptosomal preparations⁶. The enzyme activity was measured as $\mu\text{mol P}_i$ released per mg protein per 30 min. Incubation medium contained 0.03 M imidazole, 0.06 M KCl, 0.5 mM ATP; 1 mM Mg^{2+} or Ca^{2+} , 0.1 mM ouabain and 0.1 mg protein, final volume 1 ml, pH 7.2. Samples were preincubated 10 min at 37° C without ATP, in the presence or absence of various concentrations of cytochalasin B. Controls contained equal amounts of dimethylsulphoxide. The data were expressed as the percentage of control activity. Each point is the average of duplicate determinations. ○, Ca^{2+} ; ●, Mg^{2+} . *b*, Inverse of rate of enzyme activity against cytochalasin B concentration (CB).

B inhibited the Mg^{2+} -stimulated adenosine triphosphatase activity approximately 50%. This was equally true for myosin plus actin, myosin plus neurin, stenin plus actin and stenin plus neurin.

These studies clearly demonstrate that cytochalasin B inhibits the Mg^{2+} -stimulated adenosine triphosphatase activity of reconstituted muscle actomyosin as well as that of the analogous protein from nerve ending preparations. The effect on the Mg^{2+} -stimulated but not on the Ca^{2+} -stimulated enzyme activity suggests that the action of the drug is on the interaction between actin and myosin. It has been suggested that cytochalasin B disrupts actin-like microfilaments¹. Although Wessels *et al.*¹ found no effect of cytochalasin B on the ability of striated muscle actomyosin to 'super-precipitate', Spudich and Lin⁹ did find that this compound caused a decrease in the viscosity of the actomyosin complex, as well as of F-actin, and inhibited the adenosine triphosphatase activity of the actin-heavy meromyosin complex; they interpreted their findings as indicating that cytochalasin B interacted with actin. Smooth muscle contraction and cardiac muscle contraction have also been described as sensitive to cytochalasin B¹ as is the fibrillogenesis of developing muscle cells of chick embryos¹⁶. The drug also reversibly stops the beating of the developing chick heart¹⁶.

We have proposed⁷ that the process of exocytosis by which transmitter material is released at synaptic endings is in part attributable to a chemomechanical interaction between stenin associated with presynaptic storage vesicles and neurin associated with the presynaptic membrane or neurofilaments.

The effects of cytochalasin B on the K^+ -stimulated release of noradrenaline and on the Mg^{2+} -stimulated adenosine triphosphatase activity of brain actomyosin-like protein give further support to this proposal.

This research was supported in part by Public Health Service grants and the Clinical Research Center for Parkinson's and Allied Diseases. We thank Miss Irene Tar and Mrs Stella Lamme for technical assistance.

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Skin Temperature Modifies the Pleasantness of Thermal Stimuli

MUCH of human temperature regulation is mediated by behaviour, and thermoregulatory behaviour is guided in large measure by thermal sensations, particularly their affective components, that is, their pleasantness or unpleasantness. When we feel unpleasantly cold or warm, we turn the thermostat up or down, put a sweater on or take it off.

The thermal environment that results from regulatory activity is one that the person finds pleasant, or at least not unpleasant. What a chosen environment will be, that is, at what skin temperatures the person will feel comfortable, depends significantly on internal body temperature. The same temperature on a portion of the skin can seem pleasant or unpleasant given different internal temperatures¹. It seems likely, however, that much behavioural thermoregulation takes place at constant internal temperatures^{2,3}. It would be disadvantageous to wait until internal temperature rises or falls before a person takes appropriate action.

Given this line of reasoning, we predict that pleasantness and unpleasantness of thermal stimuli should depend on the temperature of the skin before stimulation—which itself reflects environmental conditions—given constant internal body temperatures. Yet some observations^{1,4} suggest that pleasantness and unpleasantness depend primarily, if not solely, on internal temperature. Cabanac¹ found that ratings of affect produced

by warming and cooling the hand to various temperatures was independent of mean skin temperature, which was controlled by immersing the entire body in water. In the study reported here, we have shown that affective responses do depend on superficial skin temperature, at least when it is varied by changing the temperature of ambient air, and a portion of the subject's skin is selectively stimulated by radiation. Thus our data agree with the familiar behavioural response of people who light fires in the winter, but not in the summer, to heat their hands and faces.

We applied the method of magnitude estimation⁵ to scale the perceived affective intensity of transient (3-s) exposures of 21.8 cm² of the forehead to thermal radiation. Stimuli were presented in random order, at the rate of one every 45 s. Subjects stated whether each stimulus was pleasant or unpleasant, then assigned a number in proportion to the degree of pleasantness or unpleasantness. Subjects were instructed to assign numbers of equal magnitude to stimuli that produced equal degrees of pleasantness and unpleasantness. Each subject's forehead was blackened with India ink to promote absorption of the radiation, which was produced by a quartz lamp. A thermocouple monitored the temperature of the portion of the forehead that was stimulated, and, in two of the subjects, internal (oesophageal) temperature was measured continuously with a thermocouple-tipped catheter. Both thermocouples were copper-constantan junctions. The oesophageal thermocouple was inserted at the level of the heart: oesophageal temperature closely follows the temperature of the blood⁶. Five young men served as subjects. In all test sessions, they were dressed in light-weight clothes: however, in sessions run at low ambient temperatures (5° and 10° C), they wore a laboratory coat over their clothes.

Figure 1 shows, for two of the subjects, how mean magnitude estimates relate to both stimulus irradiance and rise of skin temperature; these results are typical of all five subjects. Local sensation on the forehead to the ambient air was close to neutral in affect when no heat was applied. As stimulus irradiance increased, the pleasantness of the sensation increased to a maximum, and then declined to neutrality, after which the sensation became more and more unpleasant. The exact shape of the curve depended strongly on ambient air temperature: when ambient temperature was low (5° and 10° C), pleasantness increased to relatively high values, and the sensation became unpleasant—though not painful—only at high stimulus intensities.

All test sessions lasted less than 40 min, sessions at the extreme temperatures (5° and 30° C) less than 30 min. The

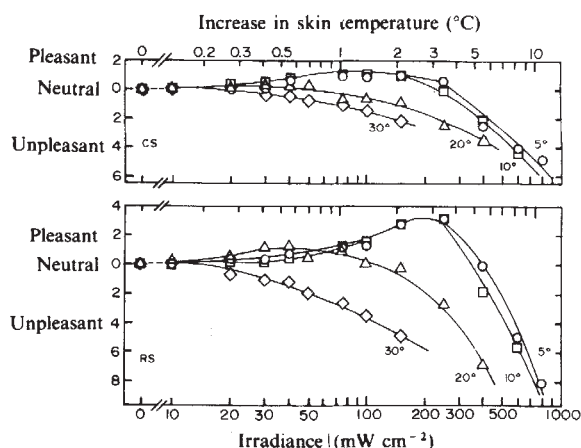


Fig. 1 Average magnitude estimates of pleasantness and unpleasantness as functions of both stimulus irradiance (lower abscissae) and resulting increase in skin temperature (upper abscissae). The parameter is air temperature. Each point is the average of two or three judgments per session made during two or three sessions.

use of brief sessions made it unlikely that internal body temperature would have time to change; measurements taken with the oesophageal thermocouple on these two subjects confirmed the constancy of internal body temperature at $37^{\circ} \pm 0.2^{\circ}$ C.

Clearly, the affective responses of the thermal system are determined in part by signals arising from the skin. The lower the ambient temperature, the lower the initial skin temperature and the greater the rises in skin temperature that are perceived as pleasant. Cabanac¹ found that the affective responses varied with both temperature of the stimulated skin and internal body temperature, but not at all with mean skin temperature. In the experiment described here affective responses did depend on the temperature of the skin before stimulation. A decrease in the ambient temperature from 20° to 5° C resulted in a decrease of forehead temperature by only 1° C for subject C. S. and 2° C for subject R. S. But the curves in Fig. 1 obtained at 5° C air temperature are displaced more than 1° and 2° from curves obtained at 20° C. Our results demonstrate, therefore, that affective responses vary with both pre-stimulus temperature of the skin and the temperature of the skin produced by radiation. An increase of skin temperature to 34° C, for example, was felt as very pleasant in cold air, but unpleasant in warmer air. The difference between our results and those of Cabanac¹ may be due to use of radiant rather than conducted heat or to different affective responsivity of the forehead and hand.

Even in constant environmental conditions, the affective dimension of thermal sensation is not determined solely by the rise in temperature produced by heat. We also found that pleasantness and unpleasantness display spatial summation. An example of results is shown in Fig. 2. When areas of 3.68 and 21.8 cm² of the forehead were stimulated alternately in the same sessions, it became clear that size of the area that is stimulated counts as importantly as how much heat is applied. Warmth sensation itself displays generous spatial summation, in that over low and moderate stimulus irradiances, doubling the stimulated area is about as effective as doubling the irradiance^{7,8}. As Fig. 2 shows, spatial summation is also a prominent feature of thermal affect.

We conclude therefore that the affective dimension of thermal sensations produced by transient stimulation is determined at

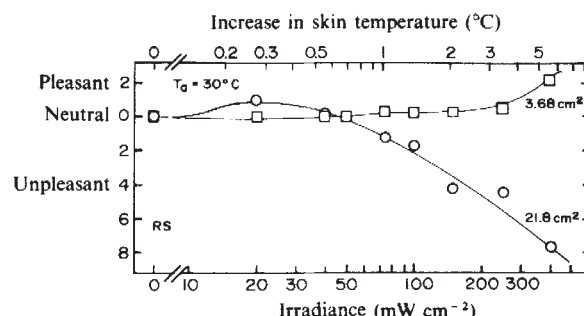


Fig. 2 Average magnitude estimates of pleasantness and unpleasantness as functions of both stimulus irradiance (lower abscissa) and resulting increase in skin temperature (upper abscissa). The results depend strongly on the extent of the area stimulated. Each point is the average of two judgments per session made during three sessions.

least in part by the thermal signals integrated over the skin. Our results are consistent with an extension of Cabanac's hypothesis^{1,9} that affect tends to improve or restore homeostasis. The ambient temperatures 5° and 10° C that we used would, under long exposure, lower internal temperature. The affective responses to transient heating seem, then, to be anticipatory to the extent that they are homeostatic; Gagge *et al.*¹⁰ noted this anticipatory component to transient thermal stimuli. Heat in the cold is pleasant, and, to some extent, enough of it helps to keep internal body temperature from

falling. But thermal homeostasis may not be the whole story. High-irradiance stimulation is perceived in cold air as unpleasant, even though it does not suffice to make up for heat that has been lost. This perception of unpleasantness may serve to warn the organism of possible pain, and tissue destruction that will result if the heat stimulus continues.

This research was supported by the US Public Health Service.

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Received September 21; revised November 6, 1973.

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Release of Lysosomal Enzymes by Alveolar Mononuclear Cells

THE finding that individuals severely deficient in the circulating enzyme inhibitor α_1 -antitrypsin usually contract panlobular emphysema at an early age suggested that proteolytic enzymes were involved in the connective tissue destruction characteristic of pulmonary emphysema¹. This idea was strengthened by reports that intratracheal administration of the relatively non-specific enzyme, papain, or the more specific enzymes, elastase or collagenase, led to the development of emphysema-like lesions in a variety of animal species^{2,3}. The origin of such proteolytic enzymes *in vivo*, and their mode of release has been the subject of much speculation, and two cell types, the polymorphonuclear (PMN) leukocyte and the alveolar macrophage are implicated. Evidence favouring the PMNs include their sequestration in the basilar portions of the lung in the non-diseased person, their containing large numbers of lysosomes, and the high incidence of concurrent infection (with resultant leukocyte infiltration) associated with non α_1 -antitrypsin related emphysema. In addition human PMNs release approximately 25% of their lysosomal enzymes during phagocytosis of various particles such as zymosan, latex, and immune complexes⁴.

Evidence also exists favouring the involvement of alveolar macrophages in the pathogenesis of emphysema: these cells contain larger amounts of certain lysosomal enzymes⁵, have an elastase which is not inhibited by α_1 -antitrypsin⁶, and are present in the lung in high concentrations during infectious and non-infectious states. Rats exposed to low levels of NO₂ develop emphysema-like lesions without infiltration of polymorphonuclear leukocytes⁷. Peritoneal macrophages have recently been shown to release approximately 15% of their enzyme content during phagocytosis⁴. To determine if the alveolar cells likewise release a portion of their lysosomal enzymes during phagocytosis mononuclear cells lavaged from the guinea pig lung were incubated with zymosan particles, and the concentrations of β glucuronidase and elastase, lysosomal enzymes, and lactate dehydrogenase (LDH), a cytoplasmic enzyme which leaks from dying and dead cells, were monitored in the suspending media and cells. Selective release of lysosomal enzymes occurs when proteolytic enzymes, but not LDH, are found in the media.

Male Hartley strain guinea pigs, 250–300 g were injected intracardially with 0.2 ml complete Freund's adjuvant (Fisher) to stimulate production of alveolar mononuclear cells⁸. Two weeks later the animals were anaesthetised with sodium pentobarbital, 40 mg kg⁻¹, and exsanguinated from the jugular vein. Blood was allowed to clot for 2 h at room temperature and the serum collected. The thoracic cavity was opened and the pulmonary artery perfused with saline until the pulmonary circulation was free of blood. The heart and lungs were removed, blood washed from their surfaces, and the lungs lavaged with saline until 110 ml was collected. After collection the cells were counted, centrifuged, and resuspended in Medium 199 (Microbiological Associates) at a concentration of 10⁶ cells ml⁻¹.

During the cell collection and preparative manipulations, zymosan particles (Nutritional Biochemical Company) were

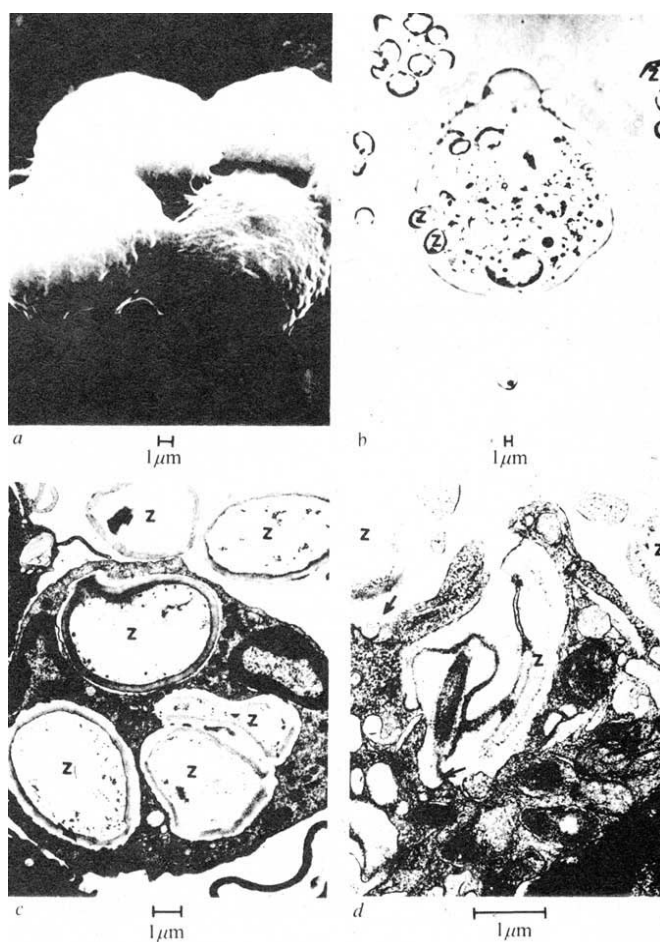


Fig. 1 *a*, Scanning electron micrograph of guinea pig alveolar mononuclear cells. Cells were fixed in 2.5% glutaraldehyde, washed in distilled water, air dried, coated with gold-palladium to a depth of 200 Å, and examined with a Jeol Scanning Electron microscope at an accelerating voltage of 25 kV. Note differences in the sizes of cells and the degree of roughness of the external membranes. *b*, Alveolar mononuclear cell phagocytosing zymosan particles (Z) as seen with phase contrast microscopy. A large amount of granular material is scattered throughout the cell. *c*, Transmission electron micrograph of alveolar cells incubated with zymosan (Z). Cells were fixed with buffered glutaraldehyde, 2.5%, post-fixed in osmium tetroxide, stained with uranyl acetate in 50% ethanol, embedded in epoxy resin, and examined with a Siemens Electron Microscope. Electron dense material is present within the phagolysosomes (arrows). *d*, Transmission electron micrograph of alveolar cells and zymosan. A phagocytic vacuole apparently open at the cell periphery with electron dense material being extruded into its internal border ('regurgitation during feeding'). At the interface between a zymosan particle (Z) and the cell a lysosome is releasing its contents directly to the outside (reversed endocytosis).

Table 1 Effects of serum and gaseous environment on β glucuronidase and LDH release from alveolar mononuclear cells

Conditions	n†	β Glucuronidase		P‡	Enzyme release*		P	Percentage uptake§ (mean $\pm$ s.d.)
		Incubation without particles (mean $\pm$ s.d.)	Incubation with particles (mean $\pm$ s.d.)		Incubation without particles (mean $\pm$ s.d.)	Incubation with particles (mean $\pm$ s.d.)		
Cells+isologous serum in 95% O ₂ -5% CO ₂	6	8.8 $\pm$ 2.0	26.6 $\pm$ 2.7	<0.01	7.0 $\pm$ 1.3	9.5 $\pm$ 0.6	<0.2	89.3 $\pm$ 1.4
Cells+isologous serum in air	6	11.8 $\pm$ 2.3	17.3 $\pm$ 2.3	<0.2	7.3 $\pm$ 1.0	10.2 $\pm$ 1.9	<0.2	86.2 $\pm$ 2.9
Cells+autologous serum in 95% O ₂ -5% CO ₂	6	8.3 $\pm$ 2.0	26.3 $\pm$ 2.4	<0.01	7.0 $\pm$ 2.0	7.7 $\pm$ 1.3	<0.8	92.6 $\pm$ 0.4

* β Glucuronidase and lactate dehydrogenase values are the percentage of total enzyme released into the supernatant during a 2 h incubation period under the specified conditions. Cell levels were 1.49 ± 0.59 nM β glucuronidase per million cells per min and 141 ± 43 absorbancy units LDH per million cells (mean $\pm$ s.d., twelve experiments each).

† Number of experiments.

‡ Probability as determined by Student's *t* test.

§ Percentage of cells containing two or more zymosan particles.

incubated for 30 min with fresh serum previously obtained. Ten million cells in Medium 199, a 0.5% solution of zymosan, and 10% fresh guinea pig serum (final volume 2 ml) were incubated for 2 h in a Dubnoff shaker, 37° C, and gassed with either air or 95% O₂-5% CO₂. The mixtures were immediately placed on ice, centrifuged at 500g for 10 min and the supernatants assayed for β glucuronidase, elastase, and LDH. One aliquot of cells was resuspended in acetate buffer, 0.1 M, pH 4.5, containing 0.1% Triton X-100 (Sigma) and sonicated with an E/MC Ultrasonic cleaner oscillating at 90 Hz s⁻¹ and assayed for β glucuronidase activity using a phenolphthalein glucuronide (Nutritional Biochemical Company) substrate⁹. Another aliquot was suspended in the same buffer without Triton X-100, sonicated for 10 min, and assayed for elastase using *t* boc-l-alanine nitrophenyl ester (Cyclo) substrate in phosphate buffer, 0.05 M, pH 6.5 (ref. 10). The elastase activity determined after sonication without Triton X-100 was equivalent to that found after ten freeze-thawing cycles indicating that the lysosomes had been cleaved by the sonication procedure. LDH levels were determined by measuring the initial rate of oxidation of NADH₂ (Sigma) in the presence of 0.01 M sodium pyruvate¹¹.

The degree of phagocytosis was ascertained by counting the number of cells which had engulfed at least two zymosan particles. A minimum of 100 cells was examined.

The cells were viewed under phase, transmission, and scanning electron microscopy. Over 80% were monocytes or macrophages, approximately 15% were lymphocytes, and the remainder were eosinophils. Few if any basophils, neutrophils, or erythrocytes were found in the preparations. When viewed under scanning electron microscopy virtually all cells had the ruffled membranes typical of phagocytic cells (Fig. 1a).

Table 2 Release of elastase from alveolar mononuclear cells exposed to zymosan particles*

	n†	Elastase activity (ΔA per 30 s)		P‡
		Incubation without particles (mean $\pm$ s.d.)	Incubation with particles (mean $\pm$ s.d.)	
Cells	4	0.0143 $\pm$ 0.0022	0.0060 $\pm$ 0.0011	<0.01
Supernatant	4	0.0175 $\pm$ 0.0064	0.0160 $\pm$ 0.0061	n.s.
Serum	4	0.0187 $\pm$ 0.0069	0.0170 $\pm$ 0.0050	n.s.

* Ten million alveolar mononuclear cells, 0.5% zymosan, and 10% fresh isologous serum (final volume, 2 ml) were incubated for 2 h with 95% O₂-5% CO₂ gas mixture. The cells were centrifuged, resuspended in 2 ml 0.05 M sodium acetate buffer, pH 4.5, and sonicated. 0.2 ml of the supernatant solution and 0.2 ml of the sonicate were assayed for elastase activity with *t* boc-l-alanine nitrophenyl ester as substrate.

† Number of experiments.

‡ Probability as determined by Student's *t* test.

Approximately 90% of the cells phagocytosed the zymosan

particles, and the uptake was independent of the gaseous environment or serum sources employed (Table 1; Fig. 1b and c). Those cells incubated with the zymosan, in either isologous or autologous sera, and in the presence of 95% O₂-5% CO₂, released more than three times as much β glucuronidase as the cells similarly treated but not exposed to the particles. Cells incubated with the particles in air released only slightly more β glucuronidase than the controls. Alveolar cells are continually exposed to a highly oxygenated environment *in vivo*, use more oxygen than either peritoneal macrophages or PMNs¹², and probably depend on an oxidative energy metabolism for phagocytic function and its sequelae, enzyme release.

Three mechanisms have been proposed to account for enzyme release from phagocytic cells. The first, initially described by Metchnikoff, involves the release of material from dead or dying cells. Release in this instance is 'non-selective', that is, cytoplasmic and lysosomal contents would be expelled concomitantly. Approximately 7% of the total LDH was found in the supernatants from cells not exposed to the zymosan; this percentage was enhanced by the presence of zymosan, especially when the environment contained air, but in no cases were the differences in LDH release between cells incubated in the presence and absence of zymosan statistically significant. The release, then, was selective in that the lysosomal but not the cytoplasmic enzyme marker was extruded from cells exposed to zymosan.

The second mechanism, termed reversed endocytosis, occurs when the lysosome migrates to the surface of the cell and expels its contents directly to the outside¹³. Such a pattern is normally observed when cells are exposed to non-phagocytosable surfaces¹⁴, or to a phagocytosable substance in the presence of cytochalasin B¹⁵. The third mechanism, 'regurgitation during feeding', is characterised by the release of enzyme through the open end of an incompletely fused phagolysosome¹⁶. In Fig. 1d a cell demonstrating both reversed endocytosis and regurgitation during feeding is presented.

A second lysosomal enzyme, elastase, may be especially important in the pathogenesis of emphysema. Elastin isolated from emphysematous human lungs had significantly decreased ratios of non-polar/polar amino acids suggesting that the outer core of elastin, which is thought to be composed of the non-polar amino acids, was degraded by locally released elastase. The alveolar walls of rats exposed to the proteolytic enzyme thermolysin showed fragmentation, thickening, and degeneration characteristic of emphysema as well as a decrease in the percentage of non-polar amino acids¹⁷. In our experiments the elastase found in the supernatant from cells incubated with or without zymosan could be accounted for by the enzyme present in the serum; those cells incubated with zymosan, however, had significantly decreased levels of elastase (Table 2)

indicating that the enzyme was released but was either rapidly inactivated¹⁸ or adhered to the cell membrane¹⁴.

The results of these experiments provide evidence that alveolar mononuclear cells can release proteolytic enzymes on phagocytic stimulation. These cells normally engulf particulate matter that passes the 'mucociliary escalator' which lines the upper respiratory passages and may release a portion of their enzyme load directly on the bronchiolar and alveolar surfaces. Under normal conditions the α_1 -antitrypsin found in the lungs would be expected to inactivate the enzymes. In its absence, such as in the homozygous α_1 -antitrypsin deficiency, or when the amount of particulate matter is excessive, sufficiently high levels of proteolytic enzymes may be free to cleave the pulmonary connective tissue.

We thank Dr J. Sardinas and K. Massaro for the scanning electron micrographs, Dr G. Schidlovsky for the transmission electron micrographs and Dr J. Constantine for his comments on the manuscript.

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Received October 16, 1973

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immunoglobulins can be isolated on an immunosorbent from a proteolytic digest of the lymphocytic surface³. The availability of immunosorbents of high quality⁴ has extended the scope of such methods.

I have investigated the feasibility of evoking an antibody response by direct injection of immunosorbent with its attached antigen. The aims are to minimise losses of the antigen by stopping the preparation at the stage of immunoadsorption, and to present what little antigen there is in an optimally immunogenic form. The principle of injecting antigen adsorbed to a specific solid phase is not new⁵, but it has not been extended to the very flexible immunosorbents nor used with the small amounts of antigen often available, for example, from mammalian cell surfaces. The major apparent limitation is that to deal with an antigen having determinants ABC, an antiserum reacting with one or more of the determinants is needed initially (that is an antiserum to a cross-reacting antigen) to prepare the immunosorbent.

As a model I have chosen a requirement to raise antibodies to the V region^{6,7} of the Faby fragment from a human myeloma protein, given first, a solution containing the Faby fragment at 1 $\mu\text{g ml}^{-1}$ plus a contaminating protein, bovine serum albumin (BSA), at 1 mg ml^{-1} , and second, a rabbit antiserum to normal Faby, which by its nature will react with determinants on the C region but not the V region of the myeloma Faby.

The solid phase chosen was microcrystalline cellulose (catalogue number 2330; E. Merck, Darmstadt). This has a quoted particle size of 3 to 30 μm , but in fact a suspension in 0.1 M NaCl, 0.02 M Tris-HCl, pH 8.0, was seen to have many particles also between 1 and 3 μm . At several of the handling stages, particularly after activation with cyanogen bromide, it was seen to be aggregated, but this was reversible. It was chosen in preference to solids more commonly used for immunosorbents (Sephacrose, Sephadex, coarser celluloses) because it has many particles of a size permitting phagocytosis. Whether this potential advantage is important in practice has not yet been tested.

Immunosorbent was prepared by coupling IgG (50 mg), from the rabbit anti Faby serum, to microcrystalline cellulose (1 g) at pH 6.5, using the cyanogen bromide method^{8,9}. About 60% of the protein was coupled. In terms of packed volume of cellulose per volume of suspension, the most convenient measure of concentration, this yielded 55 ml of 5% immunosorbent. A blank immunosorbent was prepared by substituting IgG from non-immune serum in the above procedure.

In a typical experiment, 1 ml of 5% anti-Faby immunosorbent was added to 10 ml of the Faby-BSA solution and stirred constantly for 24 h at 4° C. 65% of the Faby was bound. Four washes with 0.1 M NaCl, 0.02 M Tris-HCl, pH 8.0, were carried out by displacement filtration in tubes previously described¹⁰. Previous adsorption with blank immunosorbent made no difference: as judged by immunofluorescence the blank had bound neither Faby nor BSA. (It should be emphasised, however, that in many situations it is vital to carry out blank immunoadsorption first to remove material adhering non-specifically to the matrix. This is especially important when dealing with cell lysates.) The test immunosorbent, when examined by immunofluorescence, was strongly positive for Faby but negative for BSA.

Rabbits were immunised with 0.01 ml packed immunosorbent bearing approximately 1 μg bound Faby. This was divided into equal primary and secondary doses given 5 weeks apart. Each dose was in turn divided into four subcutaneous injections, one into each shank. Table 1 compares the results obtained using various adjuvants. Freund's complete adjuvant (FCA) clearly gave the best antibody titres. No precipitins to BSA were detected in any of the sera. All subsequent experiments used FCA.

Injection into the footpads was not obviously more effective and it caused considerable inflammation. It was better to divide 1 μg of antigen into two doses given 5 weeks apart

Immunisation with Antigen coupled to an Immunosorbent

IN attempts to produce antisera to surface components of cells one is often frustrated by the fact that many components are poorly immunogenic *in situ*, presumably due to antigenic competition from myriad adjacent structures. The alternative approach of preparing a component in pure form in an amount sufficient for immunisation schedules can be formidable. Advantage may be taken, however, of an intermediate stage in some schedules for purifying such components: specific adsorption onto a solid phase. For example, the receptors for con A and phytohaemagglutinin have been isolated in this way from detergent lysates of lymphocytic plasma membranes^{1,2}; and the Fab fragments of surface

than to give it all as a single dose: this in spite of the fact that the antigen depots might be expected to yield antigen for some weeks. The antibody titre was maximal at about 2 weeks after the second dose.

The antibody response seemed to be divided approximately equally between the C and V regions of the Faby. It had been hoped that the V regions, projecting outwards, would

Table 1 Antibody responses to bound Faby

Adjuvant and dose *	Antibody titre 2 weeks after secondary injection †
Nil	0 to 2
Freund's complete adjuvant (Difco), 50% of injected volume	4 to 16
Al (OH) ₃ (Alhydrogel), 200 µg of Al	2 to 4
Polyacrylate (Primafluc AlO), 200 µg	1 to 4
<i>B. pertussis</i> , 2 × 10 ⁹ organisms	1 to 2

* The total dose of Faby was 1 µg, bound to 0.01 ml packed immunosorbent. This was mixed with saline and adjuvant to give a volume sufficient for primary and secondary lots of four subcutaneous injections, each 0.3 ml. The dose of adjuvant shown is the total for the course, divided equally among all the injections.

† Range of titres observed in three or four rabbits. Given as the reciprocal of the greatest of doubling dilutions giving a precipitin line on an Ouchterlony plate with 5 µg antigen; the reagent wells were of 6 mm diameter with their centres 10 mm apart. A strong precipitating antiserum can give a titre of 256 by this method.

provoke the stronger response. Previous sensitisation to the C regions, by subcutaneous injection of 0.1 mg normal Faby in FCA 1 week before the primary injection, diminished the response to the V regions. An attempt to make the animals tolerant to the C regions by giving 50 mg monodisperse IgG intravenously at the time of the primary injection failed. However passive antibody^{11,12} to the C regions (60 mg IgG from the rabbit antiserum to normal Faby, given intraperitoneally on the day following the primary injection) markedly diminished the response to the C regions and probably enhanced that to the V.

It is clear that the procedure described is capable of giving moderate antibody responses to minute amounts of bound antigen. I am continuing to evaluate the variables involved.

This work was supported by Tenovus of Cardiff and the Wessex Regional Hospital Board. We thank Mr P. A. Knight of Wellcome Research Laboratories for advice on adjuvants and Mrs Maureen Power and Mr R. Chapman for technical assistance.

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Received October 29, 1973.

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Dual Action of Praseodymium (Pr³⁺) on Transmitter Release at the Frog Neuromuscular Synapse

THE release of transmitter after a nerve action potential seems to require the entry of calcium ions through the presynaptic membrane^{1,2}. Mitochondrial uptake within the nerve terminal constitutes a possible way of removing these calcium ions temporarily^{3,4}. While inhibition of the first process, namely the entry of calcium, would be expected to decrease the mean number of quanta liberated by a nerve impulse (quantal content, *m*), selective inhibition of the second process would tend to augment the release of transmitter.

A substance that acts on both calcium transport processes, may have either an inhibitory or facilitatory effect, depending on the relative sensitivities of these two processes and the appropriate concentrations. Lanthanides are known to be strong inhibitors of calcium uptake by the mitochondria⁵, and lanthanum (La³⁺) is known to prevent entry of calcium ions into nerve^{6,7}. We have attempted to distinguish between these two opposing actions using the lanthanide praseodymium (Pr³⁺).

We used superficial fibres of the cutaneous pectoris nerve muscle preparation of the frog (*Rana pipiens*). Intracellular electrodes (3 M KCl, 40–90 MΩ resistance) were inserted into the muscle, usually employing Nomarski interference optics. The penetrations were made 200–300 µm from the nerve terminal. The preparations were kept in Ringer solution of the following composition: 116 mM NaCl, 2.0 mM KCl, 1.8 mM CaCl₂, 0.25 mM NaH₂PO₄. To block transmission and thereby prevent contractions, 10–12 mM MgCl₂ was added to the medium by isotonic substitution for sodium. Spontaneous activity (miniature end-plate potentials, m.e.p.p.) and evoked potentials (end-plate potentials, e.p.p.) were recorded conventionally.

Addition of 200 µM Pr³⁺ or more to the perfusion medium caused a rapid abolition of the e.p.p. (Fig. 1a). This effect of Pr³⁺ corresponds to that described for La³⁺ at the neuromuscular junction^{8,9}. Another similarity of the action of these two lanthanides is the increase in spontaneous release: even at low concentrations, praseodymium causes a marked

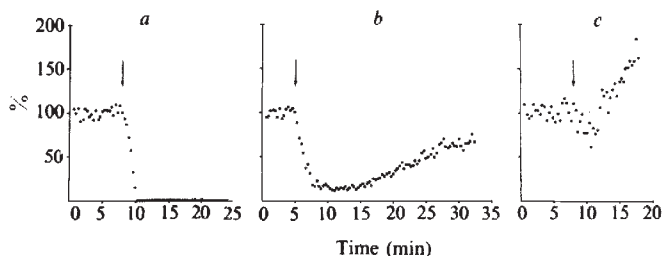


Fig. 1 The action of different Pr³⁺ concentrations on e.p.p. amplitude. Each point is the average value of 10 e.p.p.s elicited at a rate of 0.5 s⁻¹. Bathing medium contained standard Ringer with 10 mM MgCl₂ to prevent contractions. Each experiment was performed on a different muscle. All values were normalised to mean initial control value of the end plate potential, *R* (given in parentheses below). *a*, The action of 250 µM PrCl₃ (control mean value *R*=5.54 mV, *m*=19.07); *b*, 100 µM PrCl₃ (*R*=5.86 mV, *m*=23.63); *c*, 15 µM PrCl₃ (*R*=1.84 mV, *m*=5.36). All experiments were done at room temperature (20°–23° C).

increase in the frequency of the spontaneous m.e.p.p.s (Fig. 2 *b*, *d* and *e*).

However, at concentrations of 100 µM or less, the effect of

Pr^{3+} on e.p.p. amplitudes was not monotonically inhibitory: there was an initial decrease followed by an increase (Fig. 1b and c). Between 10 and 30 μM (Fig. 1c) the amplitude of the e.p.p. regularly increased beyond the initial control value

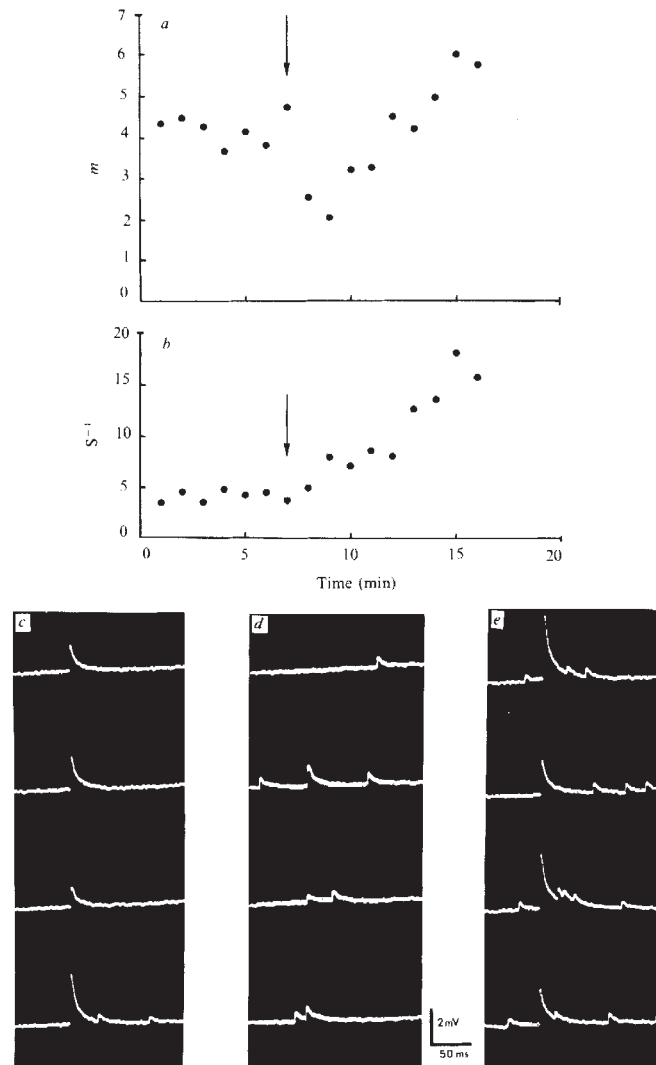


Fig. 2 Biphasic action of 15 μM PrCl_3 on evoked transmitter release. *a*, Effect on quantal content (m). Note the "dip" in m . Each point represents the mean value of twenty responses. No failure of transmission was observed in 140 responses elicited before addition of Pr^{3+} . *b*, Effect on m.e.p.p. frequency. Note that the effect on spontaneous release does not show a "dip". *c*, Sample records before the addition of PrCl_3 . *d*, Sample records 3 min after addition of 15 μM PrCl_3 . Note failure of transmission in upper trace. *e*, Sample records 8 min after PrCl_3 . Stimulation frequency 0.33 s^{-1} . All records are taken from the same fibre. Other experimental details as in Fig. 1.

during this late phase. An analysis of these responses reveals that the principal effect of Pr^{3+} is to reduce—and subsequently increase—transmitter output. Figure 2 shows that the early and late effects on e.p.p. amplitudes are reflected in corresponding changes in m , the number of quanta liberated by the nerve impulse.

We interpret these results as follows. Addition of Pr^{3+} causes a decrease in the Ca^{2+} influx induced by the action potential. This will account for the abolition of the e.p.p. at high Pr^{3+} concentrations and for the moderate initial decrease

(early phase) at low concentrations. La^{3+} does not interfere with action potential invasion of the nerve terminal⁸, and it seems unlikely that Pr^{3+} acts differently in this respect (at much lower concentrations). If Pr^{3+} penetrates the nerve terminal (and there are indications that even larger molecules are able to do so^{10–12}) it will interfere with calcium sequestration by the mitochondria (and perhaps also with other calcium removal processes). Hence $[\text{Ca}^{2+}]$ will increase in the cytoplasm of the terminal, causing the observed increase in m.e.p.p. frequency^{13,14}. The evoked Ca^{2+} influx will sum with this increased internal concentration, to produce a higher quantal liberation. This secondary effect will only be observed at low levels of Pr^{3+} , when the Ca^{2+} entry sites are far from saturated by the antagonist, and significant amounts of Ca^{2+} ions still enter the nerve terminal after each action potential. Alternatively the observed increase in m may be attributed to changes in membranal ionic conductances. La^{3+} is reported to prolong the voltage dependent K^+ conductance in nerve cells^{15,16}, and would thus prolong the action potential. This effect, however, is seen at La^{3+} concentrations approximately three orders of magnitude higher than the Pr^{3+} concentrations used here. Thus we tend to favour the former hypothesis.

In conclusion, the observed effects of Pr^{3+} on evoked and spontaneous release may suggest active mitochondrial regulation of internal $[\text{Ca}^{2+}]$, and similarities between mitochondrial and membranal Ca^{2+} transport systems⁴. We also suggest that the increase in m.e.p.p. frequency caused by La^{3+} (ref. 8) and Pb^{2+} (ref. 17) is, at least partially, due to interference with mitochondrial Ca^{2+} sequestration.

We thank Dr S. W. Kuffler for helpful discussions. This work was supported by the Sloan Foundation, by a grant from the National Institutes of Health, and by a US Public Health Service international research fellowship.

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Does α -Bungarotoxin Inhibit Motor Endplate Acetylcholinesterase?

α -BUNGAROTOXIN, a specific and irreversible binding agent of acetylcholine receptors of the motor endplate^{1,2} and of other nicotinic cholinergic receptors³, has no inhibitory effect upon acetylcholinesterase preparation from electrophax of electric eel³. Štalc and Župančič⁴ have, however, reported that, when a minced preparation of mouse diaphragm was used, α -bungarotoxin as well as *d*-tubocurarine markedly inhibited the membrane acetylcholinesterase when acetylcholine concentrations lower than 12 μ M were used. They took this result as evidence to support their hypothesis that the anionic centre of the enzyme in the motor endplate is the acetylcholine receptor⁵. We attempted, therefore, to re-examine the effect of α -bungarotoxin by using intact acetylcholinesterase in the motor endplate in order to test the hypothesis and to establish the specificity of binding of the toxin.

α -Bungarotoxin isolated by fractionation on column chromatography^{6,7} was found to be slightly contaminated with acetylcholinesterase existing in the crude venom of *Bungarus multicinctus*. The toxin solution, 1 mg ml⁻¹ in acetate buffer, pH 4.0, was heated to 70° C for 20 min to completely decompose the enzyme activity. That α -bungarotoxin was not affected by this treatment was confirmed by its neuromuscular blocking action. The enzyme activity was measured on the intact isolated skeletal muscles according to the method proposed by Mittag *et al.*⁸. This procedure measures only the membrane enzyme located at the endplate. Two hemidiaphragms from each of several Long Evans strain rats of either sex weighing about 250 g, or four hemidiaphragms from each of several NIH strain adult mice, or six biventer cervicis muscles from each of several 7 to 15 d old male Leghorn chicks, were quickly isolated and immersed in an organ bath containing 10 ml Tyrode solution at 37° C and oxygenated with 95% O₂ and 5% CO₂. After 30 min incubation, 1 μ M ³H-acetylcholine (290 mCi mmol⁻¹, Radiochemical Centre, Amersham) was added and the enzymatic hydrolysis measured for a 10 min period of incubation. About 20% of the added acetylcholine was hydrolysed during this period. Aliquots (1 ml) of bath medium were passed through a column (6×25 mm) of Dowex 50 to remove unhydrolysed acetylcholine, and counted on a liquid scintillation counter. The enzyme activity was expressed as the rate constant according to the following formula⁸:

Rate constant (ml per min per muscle) = $[(R-S)V]/[N(T-A)]$, where *R* is the total increase of 'acetate' c.p.m. ml⁻¹ min⁻¹; *S*, the spontaneous hydrolysis ml⁻¹ min⁻¹ in c.p.m.; *V*, the bath volume in ml; *N*, the number of muscles; *T*, the total c.p.m. per ml of the bath media; and *A*, the 'acetate' c.p.m. at zero time ml⁻¹. After the first 10 min measurement period, the test muscle preparation was incubated with α -bungarotoxin at 1 μ g ml⁻¹ for 70 min to completely block the neuromuscular transmission and to bind most of the acetylcholine receptors⁹. Then the enzyme activity was measured again in the presence of the toxin and expressed as a percentage of the first measurement. The preparation was then washed several times with plain Tyrode solution and, after 60 min, the enzyme activity was again measured. The results summarised in Table 1 illustrate that the enzyme of rat diaphragm was quite stable over the experimental period of about 4 h. It was found that when most of acetylcholine receptors were bound with α -bungarotoxin, the enzyme activity was slightly increased instead of markedly inhibited as reported by Štalc and Župančič. Similar results were obtained for the mouse diaphragm and chick biventer cervicis muscle. The enhancement of enzyme activity by the toxin seemed to be irreversible. No defect except a slight enhancement was observed even at ten times higher concentrations of α -bungarotoxin. *d*-Tubocurarine at a concentra-

Table 1 Effect of α -bungarotoxin on endplate membrane acetylcholinesterase of rat diaphragm

Agents	<i>n</i>	Cholinesterase activity		
		Before treatment (1) (rate constant; ml per min per hemidiaphragm) $\pm$ s.e.	After treatment (2) (% $\pm$ s.e.)	After washout (3) (% $\pm$ s.e.)
Control	4	0.110 $\pm$ 0.007	101 $\pm$ 3.1	97 $\pm$ 6.4
α -Bungarotoxin, 1 μ g ml ⁻¹	5	0.102 $\pm$ 0.005	117 $\pm$ 5.5 *	117 $\pm$ 7.6
α -Bungarotoxin, 10 μ g ml ⁻¹	4	0.123 $\pm$ 0.005	114 $\pm$ 3.8 *	—
<i>d</i> -Tubocurarine, 10 μ g ml ⁻¹	3	0.104 $\pm$ 0.105	81 $\pm$ 4.8 *	96 $\pm$ 0.5

* $P < 0.05$ against control.

Control hydrolysis was measured for each hemidiaphragm before treatment. The second assay was performed in the presence of agents after 70 min incubation while the third one was after subsequent washings for 60 min. The activity was expressed as a percentage of the first assay.

tion of 1 μ g ml⁻¹, which significantly blocked the neuromuscular transmission, also showed no inhibitory effect on the enzyme. About 20% inhibition was obtained at ten times higher concentration (Table 1). The enzyme, however, was completely inactivated by 0.01 μ g ml⁻¹ echothiophate, a specific inhibitor of acetylcholinesterase.

Our results with α -bungarotoxin and *d*-tubocurarine are diametrically opposed to those of Štalc and Župančič. With their *pH*-static titration method, the sensitivity at a substrate concentration as low as 12 μ M could be unreliable. Our results, therefore, indicate that the acetylcholine receptor is not identical with the anionic site of acetylcholinesterase. Barnard *et al.*¹⁰ have shown that binding of α -bungarotoxin to the mouse diaphragm receptor did not affect the binding of diisopropylfluorophosphate. These two components of electric organs of electric eel¹¹ and torpedo¹² have been separated. The slight degree of enhancement after neuromuscular blockade by α -bungarotoxin is interesting, however. Since the enhancement was the same in extent with either 1 or 10 μ g ml⁻¹ of the toxin and was irreversible, it is likely to be related to the irreversible occupation of the receptor. It may be that the blockade of the association of acetylcholine with the receptor increases the opportunity of the acetylcholine molecules to get access to the enzyme.

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Received October 15, 1973.

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The Mechanism of Auditory Evoked EEG Responses

ANALYSIS of the electroencephalogram (EEG) recorded over 0.5–1.0 s immediately following an auditory stimulus, is a well known objective method for evaluating auditory perceptual threshold, for example, in very young subjects. The low-level and visually indistinct individual responses occur in a spontaneous EEG background; hence repeated stimuli are used and the resulting ensemble of post-stimulus EEG signals averaged at each instant of post-stimulus time (PST). An exactly similar method is widely used in studying EEG visual evoked responses and other neuroelectric phenomena¹. This averaging procedure is effective in clarifying any consistent response in the presence of spontaneous activity if identical responses are evoked by all stimuli and if the response waveform is uncorrelated with the spontaneous activity; however, neither requirement seems fully to be met in EEG evoked responses generally or in evoked-response audiometry (ERA) specifically. Correlated waveforms imply common Fourier spectral components, and it is recognised that a linear filter method like averaging could only achieve limited success in separating two such waveforms since it requires the waveforms to have different spectral distributions², in which case, averaging reduces the additive background due to components with frequency outside the spectral band in which the main power of the response waveform occurs. When all consequent improvement has been made in this way, the response waveform may still seem to be degraded by the presence of background activity within the same spectral band, and linear filter methods cannot assist further; the following remarks refer to this situation³.

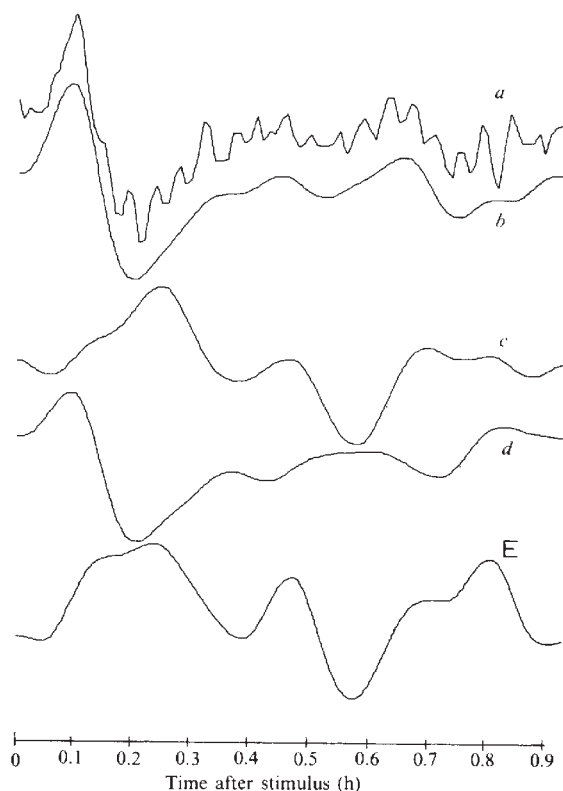


Fig. 1 Average EEG auditory-evoked responses (forty-eight stimuli, one subject). *a*, Averaged post-stimulus (response) waveform with suprathreshold stimuli (+30 dB approximately). *b*, Response waveform as in *a*, low-pass filtered to ten harmonics only. *c*, Typical averaged post-stimulus waveform obtained with subthreshold stimuli (−30 dB approximately). *d*, The waveform of *c*, phase modified according to the phase spectrum of a typical high stimulus level average response. *e*, The waveform of *c*, modified according to the amplitude spectrum of the same typical high-level response.

It is usually assumed that the ERA response consists of an additive contribution to the spontaneous activity, and this apparently unverified assumption needs examination. We have studied the ERA responses in a number of subjects using several trials with each subject at different supra- or sub-threshold auditory stimulus levels; each trial used sixty auditory stimuli such as 1 kHz tone bursts (each of 60 ms duration with 10 ms rise and decay times), with constant interstimulus interval 1.25 s, at a loudness level fixed within ± 30 dB of the approximate threshold for the stimulus used, and an ensemble of sixty signals obtained, each of 0.94 s length, starting from 50 ms after the stimulus. The analysis was effected by digital computer; the original signal was low-pass filtered to 50 Hz and sampled at 10 ms intervals with eleven-bit resolution (1 in 4,000) and all computations retain 10 ms intersample spacing. Ensemble averaging produced the averaged signal, and the characteristic response to a high level stimulus is shown in Fig 1*a* in wide band form, and Fig. 1*b* when filtered to ten harmonics. High-level stimuli thus produce post-stimulus averaged responses with clearly recognisable features.

Analysis suggests that, at least for moderate stimulus levels, the average power in the post-stimulus averaged signal, where a response can be seen, is not significantly different from that in a corresponding length of signal recorded and averaged from the immediate pre-stimulus period, where no response occurs. A similar finding applied to the comparison between post-stimulus averaged signals from the same individual using in one case high-level and in the other low-level stimuli (above or below threshold as evidenced by the appearance of recognisable features in the post-stimulus averaged signal). Hence a moderate-level stimulus that is effective in evoking an evident response apparently does not do so by contributing an obvious additive component. It therefore follows that the ERA response reflects some reorganisation of the spontaneous activity, effected by the stimulus. This could occur in either of two ways; by a redistribution amongst available spectral components of the total signal power in the spontaneous activity, or by a modification of the relative phases of the spectral components present in the spontaneous activity. The former would produce a difference between the amplitude spectra of post-stimulus averaged signals obtained with high-level stimuli and those with low-level stimuli; the latter way could produce a difference only in the phase spectra.

Amplitude and phase spectra were evaluated by digital-computer analysis of the averaged signals following effective high-level stimuli and of those following ineffective, low-level, stimuli; records from three adults and three children were used and the spectra were determined at all forty-seven harmonics of the fundamental sinusoid (1 cycle per 94 points = 1.06 Hz, fixed by the total post-stimulus signal length), and phases were calculated over the full 360 degree range. The amplitude values shown in Fig. 2 (one subject: four trials with high-level stimuli, five trials with low-level stimuli) typify the magnitudes and variation seen in all cases; neither from inspection nor from quantitative analysis can any difference be confirmed, for any harmonic, between amplitude spectral magnitudes of averaged signals that follow high-level (effective) or low-level (ineffective) stimuli. But phase spectral values (Fig. 3) do show a marked difference in distribution between signals according to stimulus level, low or high, particularly over the first ten or so harmonics. In the case of low-level stimuli, phase values for these harmonics are distributed approximately uniformly over the range (-180° , $+180^\circ$); however, with high-level stimuli, the phase magnitudes of any given harmonic of the averaged signal tend to be much less variable between trials with similarly-effective stimuli. Hence we conclude that, in these cases, effective auditory stimuli achieve a response mainly by reorganising or constraining the phase spectra of the existing spontaneous activity in the EEG.

This proposition can be tested by imposing the average phase spectral properties of responses to a high stimulus level upon the averaged signal from low-level stimuli (Fig. 1*c* shows the

original waveform filtered to ten harmonics), without altering the signal in any other way. All the spectral components of the low stimulus level averaged signal are retained without change in spectral amplitudes but their phases are those of the typical high-level response. The reconstituted waveform of the phase-modified low-stimulus-level averaged signal should then show the general features characteristic of responses to high-level stimuli typified by Fig. 1*b*. Figure 1*d* shows that this does occur. We have observed in all the cases studied so far that no other change is necessary with any of the low stimulus-level averages to transform these into an acceptable version of the high stimulus-level response, such as Fig. 1*b*; also applying this operation to any high stimulus-level response does not influence its waveform appreciably. On the other hand, if the amplitude spectrum averaged from high-stimulus level responses is imposed on the averaged signal obtained with low-level stimuli, Fig. 1*c*, without allowing any change in phase spectrum, no such effect occurs, as shown in Fig. 1*e*.

Since the averaged signal in the case of the low stimulus level apparently comprises spontaneous EEG activity alone, these results also are consistent with the above proposal that, at least with some subjects, effective stimuli act by synchronising or controlling the phases of spectral components of the spontaneous EEG activity already present. It would follow that imposing the average phase-spectral properties of high stimulus-level responses upon lengths of arbitrarily selected spontaneous EEG should also be sufficient to produce a waveform acceptably similar to a true high-level response; we have found this to be true in records of control EEG from these subjects. The effect of this phase-modification on 0.94 s lengths of spontaneous EEG (selected at 1.25 s intervals from a control EEG recorded during a stimulus-free period) is shown by the correlation coefficient, r , calculated between an individual record length of control EEG

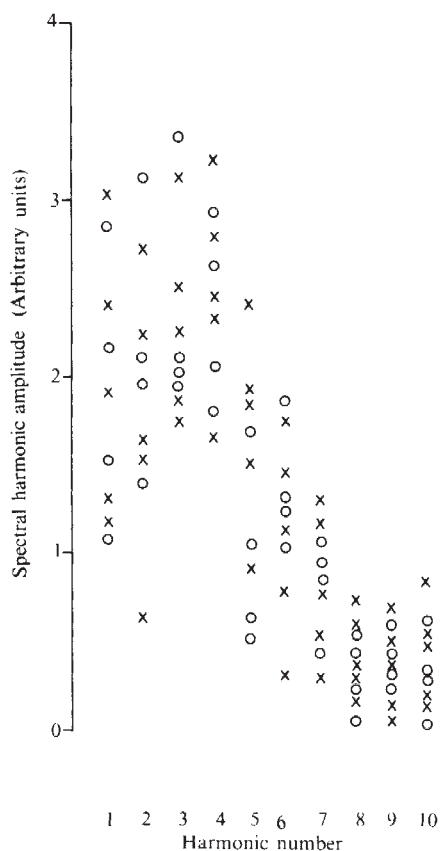


Fig. 2 Amplitude spectra of the averaged post-stimulus waveforms in nine different stimulus-level situations, four with effective, suprathreshold stimuli (○), and five with ineffective, low-level stimuli (×).

and a reference high-level averaged evoked response. Averaged over forty-eight record lengths, $\bar{r}_C = 0.01$ indicated, as expected, no correlation, but when the same forty-eight record lengths of control EEG were subjected to the phase modification described above (using the average phase spectrum drawn from the high-level responses for that subject), $\bar{r}_{CP} = 0.63$; this shows quantitatively the substantial similarity to a true high-level response achieved by appropriate phase-spectral control imposed on arbitrary lengths of spontaneous EEG in the absence of any stimulation. These figures can be compared with correlation coefficients $\bar{r}_S = 0.29$ and $\bar{r}_{SP} = 0.70$, referring respectively to the average correlation between the same reference high-level response and forty-eight individual sweeps of record following high-level stimuli first, as recorded, and second, after phase modification of each individual sweep. Comparable figures are obtained with each subject, although of course the result would have been slightly less marked if we had allowed the phase spectral values at each harmonic to be distributed randomly around the mean value. For example, if a uniform distribution is chosen, extending to ± 50 degrees from the mean, then $\bar{r}_{CP}$ (against phase-modified control EEG) = 0.43 and $\bar{r}_{SP}$ (against phase-modified stimulated responses) = 0.39.

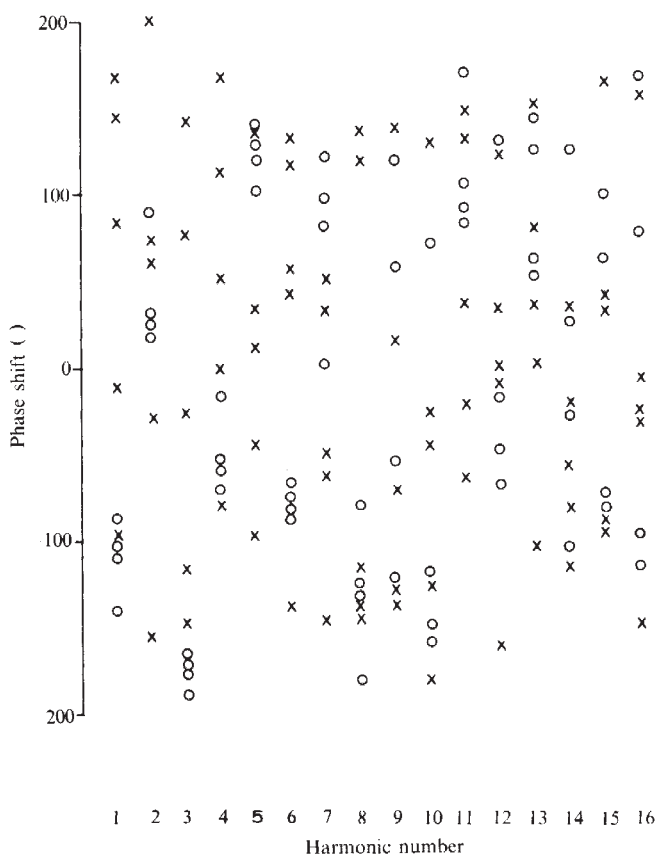


Fig. 3 Phase spectra of the waveforms as in Fig. 2.

As would be expected from these findings, it has proved possible to produce a waveform again similar ($\bar{r} = 0.69$) to the high level response of one subject, by imposing the phase control appropriate to that subject on the spontaneous control EEG from another subject drawn from our series, even when the high-level responses of the two subjects (one adult, one child) are sufficiently different as to be readily distinguishable visually and very poorly correlated ($r = 0.01$); the converse has also been demonstrated. If this finding is more generally confirmed, it follows that alternative methods of detecting the effect of a stimulus acting through such a mechanism may be more

appropriate than the usual averaging procedure; a method based on the evaluation of phase spectra in individual sweeps will be reported elsewhere. It also follows that the notion of signal-to-noise ratio (which in any case relates only to signal power, or signal amplitude) applied to a response waveform and its apparent noise background, is inapplicable to a situation in which a phase-control mechanism operates; effectiveness of a stimulus would better be expressed as the extent of phase control imposed on the spontaneous activity, and this is a readily quantifiable measure. While averaging or any other form of linear filtering will not improve the detectability of a stimulated response in the presence of additive background activity in the same spectral band, the results reported here suggesting that an effective stimulus acts mainly to phase-control the spontaneous activity, thus indicate that the approach proposed above may be fruitful. Closer attention to phase characteristics may be useful in other areas of signal processing, as for example in recognising patterns in background noise.

We acknowledge the support of the Medical Research Council, the Spastics Society, and the Ewing Foundation.

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Correlation of Behavioural Differences in Three Strains of Mice with Differences in Brain Amines

MUCH experimental evidence supports the view that brain amines may be related to behaviour, but more data are necessary regarding the relative roles of dopamine (DA), noradrenaline (NA) and serotonin (5-hydroxytryptamine) (5HT). The behavioural states have been mainly investigated using either drugs which modify catecholamine metabolism¹⁻¹² or conditioning in which stress cannot be excluded¹³.

The use of inbred strains of mice may be of particular interest for further elucidation of the neurochemical correlates and their possible relationship to acknowledged behavioural differences.

The reason for using inbred strains is the behavioural homogeneity of individuals belonging to the same strain¹⁴, in contrast to the large difference in the behavioural traits which characterise a heterogeneous population of different strains. Major differences were found in emotionality, learning, activity and aggressiveness in different strains¹⁴⁻¹⁷. Inbred strains also represent useful material in pharmacogenetic analysis of different psychoactive drugs. In spite of much comparative behavioural and psychopharmacological data, there have been few neurochemical investigations of regional brain neurotransmitter metabolism in selected strains^{15,17-21}.

The present study was performed with 2 to 3-month-old male mice of three strains C57Bl/6J, DBA/2J and SEC/

1ReJ and their F₁ hybrids obtained from Jackson Laboratory, Bar Harbor, Maine. Previous behavioural studies performed with animals of these strains of similar age and sex to those used here have shown that strain differences exist in wheel-running activity, avoidance behaviour and maze learning²².

The C57Bl strain is characterised by high spontaneous activity (304.5 wheel revolutions h⁻¹) and low levels of avoidance (6.6% avoidance in a shuttle box and 94 errors in a maze), while the two other strains (DBA and SEC) attained high avoidance levels (respectively 40 and 53.4% avoidance and 69.3 and 50.5 maze errors), but much lower general activity (respectively 230.3 and 203.6). Crossing the C57 strain with the high avoiding low running SEC mice resulted in SEC-like behaviour progeny (activity 195, avoidance 48.3%, maze learning 46.9), while crossing the C57 strain with the high-avoiding low-running DBA mice yielded offspring similar to the C57 phenotype (activity 288.1, avoidance 8.3% and maze learning 45.9). Behavioural data are from Oliverio *et al.*²².

This report concerns catecholamine metabolism in whole brain and some areas of the central nervous system in the three strains.

The mice were killed by decapitation. The total brain and the different areas (hypothalamus, pons medulla and cortex) (Fig. 1) were removed, weighed, frozen and stored in liquid nitrogen.

The tissues were homogenised in 10 vol of acid ethanol (ethanol-water-HCl 0.05 N, 74:16:10 (v/v) at 0° C in a Potter Elvehjem homogeniser and then centrifuged at 10,000g for 10 min. The amines were separated on columns of Amberlite CG50 200-400 mesh²³. Noradrenaline²⁴, dopamine²⁵ and 5HT²⁶ were estimated fluorometrically. Turnover parameters were determined by blocking the synthesis of noradrenaline and dopamine by intraperitoneal injections of α -methyltyrosine (DL- α -methyl-*p*-tyrosine methylester HCl, AB Biotec Stockholm, Sweden) (300 mg kg⁻¹) according to Brodie *et al.*²⁷. Animals were killed 1-3 h after injection. Breakdown of 5HT was blocked with pargyline (75 mg kg⁻¹) and the subsequent changes in 5HT concentrations were determined²⁸. Regression lines were calculated from the least squares fit. The results were analysed statistically by analysis of the covariance²⁹.

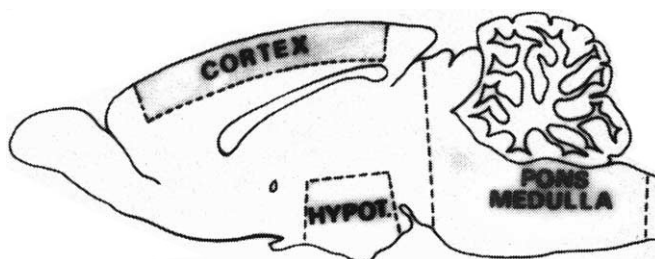


Fig. 1 Diagrammatic representation of dissection procedure for mice brain.

The weights of the mice and their brain weights are given in Table 1. The amount of NA is significantly higher in the whole brain of C57 mice compared with DBA and SEC

Table 1 Mean values for body and total brain weights in three strains of mice

	Mouse weight g $\pm$ s.d.	Brain weight mg $\pm$ s.d.
C57Bl/6J	26 $\pm$ 1.7	451 $\pm$ 17
DBA/2J	26 $\pm$ 1.5	390 $\pm$ 15 †
SEC/1ReJ	23 $\pm$ 1.2 *	427 $\pm$ 17 †

* $P < 0.02$. † $P < 0.001$.

(Table 2). After α -methyltyrosine injection (Table 2), the slope of the decrease of NA is rather close in the three strains, as is also the turnover time. Since the amount of NA is higher in the C57, the turnover rate is also slightly higher.

Table 2 Brain noradrenaline concentrations and turnover in three strains of mice

	NA (ng/gww ± s.d.)	(ng/brain ± s.d.)	After α MT (slope ± s.d.)	Turn- over time (h)	Turn- over rate (ng/g/h)
C57Bl/6J	589 ± 40 (29)	266 ± 19	0.077 ± 0.007	5.6	104.5
DBA/2J	562 ± 45* (29)	219 ± 17†	0.072 ± 0.006	6.0	93.2
SEC/1ReJ	535 ± 38† (25)	228 ± 17†	0.080 ± 0.006	5.4	98.5

* $P < 0.05$.

† $P < 0.001$.

Numbers in parentheses represent the number of mice.

In contrast to NA, the dopamine content per g whole brain are significantly greater in DBA and less in SEC compared with C57 (Table 3). When expressed in ng per brain, there were no differences between the C57 and DBA mice; only SEC presenting significantly lower values. The slope of the decrease of DA after α -methyltyrosine injection is significantly different between the three strains; as is the turnover time and the turnover rate.

Table 3 Brain dopamine levels and turnover in three strains of mice

	DA (ng/gww ± s.d.)	(ng/brain ± s.d.)	After α MT (slope ± s.d.)	Turnover time (h)	Turnover rate (ng/g/h)
C57Bl/6J	1,559 ± 189 (29)	703 ± 91	0.097 ± 0.008	4.5	348
DBA/2J	1,763 ± 216* (28)	687 ± 82	0.077 ± 0.008*	5.6	313
SEC/1ReJ	1,353 ± 193* (25)	581 ± 88*	0.137 ± 0.009*	3.2	427

* $P < 0.001$. Numbers in parentheses represent numbers of mice.

The amount of serotonin per g fresh weight is similar in the three strains (Table 4), however the quantity per brain is lower in DBA and SEC compared with C57 mice. After pargyline injection the slope of the increase of 5HT is very similar in the three strains, as is also turnover time. Since the amount of 5HT in brain is higher in C57 strain the turnover rate seems to be slightly higher in C57 mice.

When pons medulla oblongata, hypothalamus and cortex were analysed separately (Table 5), a significantly higher amount of NA was found in pons medulla in the C57 strain compared with DBA and SEC.

In contrast, less NA was found in the hypothalamus of C57 mice. In the cortical zone markedly decreased amounts were found in the SEC brain compared with C57 and DBA, the latter two having similar values.

When determining the turnover parameters of NA in the

Table 4 Brain serotonin levels and turnover in three strains of mice

	5 HT (ng/gww ± s.d.)	(ng/brain ± s.d.)	Slope ± s.d.	Turn- over time (h)	Turn- over rate (ng/g/h)
C57 BL/6J	1,041 ± 97 (22)	472 ± 45	3.21 ± 0.84	5.43	192
DBA/2J	1,016 ± 95 (22)	397 ± 38*	3.08 ± 0.84	5.50	185
SEC/1ReJ	1,017 ± 73 (18)	431 ± 26*	2.98 ± 0.83	5.67	179

* $P < 0.001$.

Numbers in parentheses represent numbers of mice.

different brain areas only the slope of the decrease of NA in the pons medulla of C57 mice is significantly different from DBA and SEC. The turnover time is much quicker in the pons medulla of the C57 mice.

In the F_1 generation (Table 6) crossing DBA and C57, values for NA in pons medulla closer to C57 were obtained, but crossing SEC and C57, values closer to SEC were observed. In the hypothalamus the NA levels were closer to those of C57 in both crosses C57 × DBA and C57 × SEC, while in the cortical area, the value from C57/DBA was lower than in the parental strain and in C57/SEC close to the value from parent SEC.

As transmitters are localised in specific areas, determination of the catecholamines in terms of ng per g whole brain may be misleading. Since the brain weight is different in the three strains seemingly high values will be obtained for animals having lower brain weights. The catecholamine levels for whole brain are more suitable for evaluating the steady state situation.

The higher levels of NA in the whole brain and in the pons medulla in the C57 strain may be correlated with the greater activity of these mice as shown in Fig. 2, *a* and *b*. Similarly, the turnover rate which is higher in C57 than in DBA and SEC may also agree with the results of the activity test. Apparently lower values of NA in pons medulla where the reticular formation is localised, agree well with the lower activity of DBA and SEC strains as well as with the activity

Table 5 Regional brain noradrenaline turnover in three strains of mice

		NA Steady-state level (ng/g)	NA decline after α MT (slope ± s.d.)	Turn- over time (h)	Turn- over rate (ng/g/h)
Pons medulla oblongata	C57	802 ± 43 (20)	0.086 ± 0.009	5.02	160
	DBA	700 ± 38† (20)	0.071 ± 0.009†	6.13	114
	SEC	740 ± 62† (14)	0.075 ± 0.007*	5.75	128
Hypo- thalamus	C57	1,468 ± 202 (20)	0.074 ± 0.011	5.88	250
	DBA	1,648 ± 178* (20)	0.073 ± 0.013	5.95	277
	SEC	1,634 ± 98* (16)	0.074 ± 0.011	5.88	277
Cortex	C57	526 ± 44 (16)	0.077 ± 0.008	5.58	94
	DBA	552 ± 71 (16)	0.082 ± 0.009	5.32	104
	SEC	375 ± 73† (8)	0.086 ± 0.013	5.03	75

* $P < 0.01$.

† $P < 0.001$.

Numbers in parentheses represent numbers of mice.

behaviour of the F_1 generation crossing either C57 and DBA or C57 and SEC (Fig. 2, *a* and *b*).

The higher values for hypothalamus NA obtained in DBA and SEC are interesting, since this area is involved in several behavioural states. A correlation with the avoidance and maze learning performance is more difficult to establish. A correlation between hypothalamic NA content and avoidance behaviour (Fig. 2c), and cortex NA content and maze learning (Fig. 2d) can be proposed, however, in the C57/

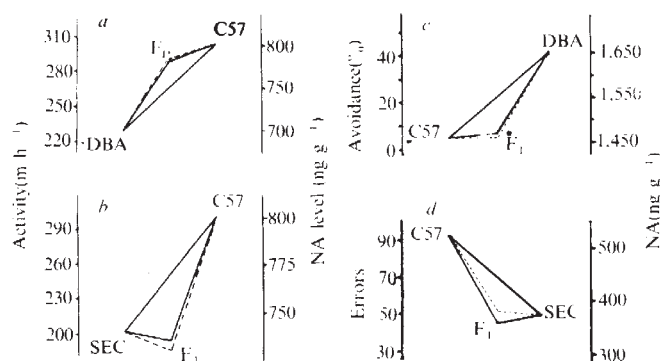


Fig. 2 Correlation between performances and NA concentrations in parental strains and their F_1 hybrids. *a-b*, Correlation between activity and NA level in pons medulla; *c*, correlation between avoidance and NA level in hypothalamus; *d*, correlation between maze learning and NA level in cortex. Unbroken lines, performances (compare Oliverio²²); broken lines, NA level in the different areas of brain.

Table 6 Noradrenaline in various brain areas of three strains of mice and their F_1 hybrids

	DBA	C57	SEC
Parental	700 ± 38	802 ± 42	740 ± 62
Pons medulla oblongata			
F_1	779 ± 43	728 ± 42	
Parental	1,648 ± 178	1,468 ± 202	1,634 ± 98
Hypothalamus			
F_1	1,473 ± 102	1,450 ± 143	
Parental	552 ± 71	526 ± 44	375 ± 73
Cortex			
F_1	470 ± 19	383 ± 39	

DBA cross for avoidance behaviour and in the C57/SEC cross for maze learning. It must be pointed out that the NA concentration in the hypothalamus of the F_1 offspring from a C57/SEC cross is identical to that of C57, whereas in the two other areas (cortex and pons medulla) the parent SEC characteristic is dominant.

It is interesting to note that for the whole brain, there is also significantly less DA in SEC, and for DBA the values are very close to C57.

Finally the whole brain 5HT content is also significantly higher in the C57 strain.

We conclude from these data that (1) the NA steady state concentrations in whole brain and some areas such as cortex, hypothalamus and pons medulla are significantly different in different inbred mice strains; and (2) heritability of NA level is similar in pons medulla and hypothalamus in the F_1 generation offspring from C57/DBA cross and these values are close to those of the C57 parent. In the F_1 generation offspring, from C57/SEC cross the NA level is close to that of the SEC parent in pons medulla and cortex but not in the hypothalamus.

Thus a correlation seems to exist between the behavioural phenotype and NA levels of cerebral regions. In particular there is a high correlation between pons medulla NA level and the activity of the C57, DBA and SEC mice and their F_1 hybrids.

We thank Mrs C. Schleaf for technical assistance. E. K. is a Chargée de Recherche au CNRS.

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Received August 29; revised October 18, 1973.

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Aerosol Chemotherapy of Lung Neoplasia

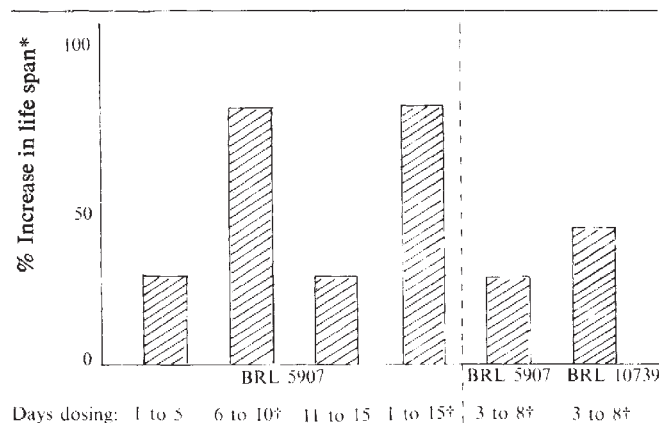
THE administration of anticancer drugs in amounts necessary to obtain a therapeutic response can be associated with severe toxicity. Attempts to circumvent such toxicity have been made and examples of this include intra-arterial infusion chemotherapy and the administration of drugs directly into neoplastic lesions. We wish to suggest a novel extension of such regional cancer chemotherapy and to describe the use of aerosol dosing to confer protective antitumour effects in the lungs of mice carrying certain transplanted tumours.

The materials used in this study were the double-stranded RNA, BRL 5907, isolated from a mycophage, and a ribonuclease resistant complex of this nucleic acid BRL 10739 (ref. 1). Solutions of these nucleic acids were nebulised into fine droplets using a Wright CF1 aerolyser (Aerosol Products

(Colchester) Ltd). The particle sizes were less than $8\ \mu\text{m}$ in diameter and the substantial proportion was about $1\ \mu\text{m}$. These droplets were therefore able to penetrate to the lower respiratory tract including the alveoli of the lungs². Mice bearing a transplantable tumour were placed in a chamber and exposed to the aerosol. The drugs, which are macromolecular and not orally absorbed, were inhaled by the mice and partly retained in the lung. The dose which was retained was calculated from the volume of the lung and respiration rate and using a 30% retention value as previously reported³. The dose can be adjusted by changes in the concentration of the drug, the flow of aerosol through the chamber and time of the experiment.

The FS6 mouse tumour, a benzpyrene-induced fibrosarcoma, was the first model to be studied⁴. FS6 tumour cells ($0.1\ \text{ml}$ trypsinised suspension of 10^5 cells) were injected by the intravenous route into syngeneic C57Bl/6 mice. The cells lodge in the capillary bed of the lung and grow to form tumours which kill the animal after three to four weeks. In the first experiment BRL 5907 was given as an aerosol at a dose of $0.4\ \text{mg}\ \text{kg}^{-1}\ \text{d}^{-1}$ to mice bearing the FS6 tumour at different times over the first 15 d after transplantation (Table 1).

Table 1 Effect of BRL 5907 and BRL 10739 by the aerosol route on the FS6 fibrosarcoma



* Calculated on day of median death in groups of ten mice.

† Increase in survival time compared with controls were statistically significant ($P < 0.01$). 10^5 cells given intravenously on day 0. Drugs given in an aerosol chamber $0.4\ \text{mg}\ \text{kg}^{-1}\ \text{d}^{-1}$. Controls were dosed with aerosol saline, median day of death 30.

An increase in survival time over the controls was found and this varied, depending upon when the drug was given. The optimum response of a 73% increase in survival time was observed when dosing from day 6 to day 10 after inoculation. Thus the median day of death in this experiment was increased from 30 d to 50 d and there were four survivors in this group of ten mice. Weaker antitumour effects were obtained if the drug was given either earlier or later. Giving the drug for 15 d was no more effective than giving it between day 6 and day 10 after tumour inoculation. In a further experiment, BRL 5907 was compared with BRL 10739 again at a dose level of $0.4\ \text{mg}\ \text{kg}^{-1}\ \text{d}^{-1}$. An increase in life span of 26% was found with BRL 5907 when dosing on day 3 to day 8 and this compared with a value of 38% with BRL 10739.

We also compared the low dose double-stranded RNA aerosol with systemic administration. Intraperitoneal doses of BRL 5907 and BRL 10739 had to be increased to $2.5\ \text{mg}\ \text{kg}^{-1}\ \text{d}^{-1}$ to show even the relatively low increase in survival times of 24% and 28% respectively when compared with controls (Table 2). It seemed that a low level of drug applied to the lung gave a better therapeutic effect in the lung than larger amounts given by a systemic route. It is difficult to give larger doses of these double-stranded RNAs

Table 2 Effect of BRL 5907 and BRL 10739 by the intranasal and intraperitoneal route on the FS6 on the fibrosarcoma

Drug	Route	Dose $\text{mg}\ \text{kg}^{-1}$	Schedule days of dosing	Increase in survival time %*
BRL 5907	Intranasal drops	5.0	3, 5, 10	53†
	Intraperitoneal	2.5	4, 8, 10, 13, 15, 17	24
BRL 10739	Intranasal drops	2.5	3, 5, 10	47†
	Intraperitoneal	2.5	4, 8, 10, 13, 15, 17	28

* Calculated on day of median death in groups of ten mice.

† Increase in survival time compared with controls were statistically significant ($P < 0.001$). 10^5 cells given intravenously on day 0.

by aerosol but it is possible to use intranasal drops. BRL 5907 ($5.0\ \text{mg}\ \text{kg}^{-1}\ \text{d}^{-1}$) and BRL 10739 ($2.5\ \text{mg}\ \text{kg}^{-1}\ \text{d}^{-1}$) given by intranasal drops on days 3, 5 and 10 after inoculation showed an increase in survival time over control FS6 mice of 53% and 47% respectively.

We were interested to confirm this aerosol activity in another tumour system. The Lewis lung carcinoma of the mouse was chosen because we could study the effect of these nucleic acids given by aerosol on the pulmonary metastases ensuing from a primary tumour present in the host⁵. The tumour was transplanted subcutaneously as small fragments and grew to some 3.0 g over 21 d. The mice were then killed, the tumour excised and weighed and the lungs examined for metastases⁶. BRL 5907 and BRL 10739 were given as an aerosol at a dose of $0.4\ \text{mg}\ \text{kg}^{-1}\ \text{d}^{-1}$ for 5 d each week to mice bearing the Lewis lung tumour.

Table 3 Effect of BRL 5907 and BRL 10739 by the aerosol route on the Lewis lung carcinoma

Drug	% tumour inhibition	Average number of lung metastases
BRL 5907	0	1.0 large 3.0 small ($P < 0.025$)*
BRL 10739	0	0.5 large 2.5 small ($P < 0.01$)*
Saline controls	—	1.0 large 10.0 small

Tumour implanted day 0, subcutaneously into the flank of groups of eight mice. Drugs dosed by aerosol $0.4\ \text{mg}\ \text{kg}^{-1}\ \text{d}^{-1}$ on days 2 to 4, 7 to 11, 14 to 18.

* The number of metastases were significantly different when compared with controls.

As expected, there was no inhibition of the primary tumour (Table 3). There was, however, a definite effect against the lung metastases and this was shown by a greater than 50% reduction in the average number of metastases in the lungs of treated animals when compared with controls. At the dose and schedule used, there was no apparent difference between the two drugs. When these drugs were given at the same dose, and on the same days, by the intraperitoneal route there was no difference between the treated and control groups. Thus, we have again shown an advantage in antitumour effects in the lung when giving low aerosol doses compared with systemic administration.

The treatment of primary and metastatic lung cancer in man with anticancer drugs is notoriously ineffectual. A possible reason for this is that the surface area of the lung is very large and the concentration of a drug at any point within the lung could be too low to deal with the malignant cells. Any attempt to raise this concentration by higher doses inevitably brings added systemic toxicity. We have demonstrated antitumour activity in the lungs of mice given low aerosol doses of antitumour drugs. We suggest that this could be a novel and useful approach to the chemotherapy of pulmonary malignant disease in man enabling high concentrations of drug to be introduced in the lung.

We thank Professor P. Alexander and Mr M. R. Boyd

for their encouragement and suggestions. We also thank Dr K. Hellmann for the Lewis lung tumour.

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Received October 11; revised November 5, 1973.

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Cumulative Cytostatic Effect of ICRF 159

THE antimetabolic action of ($\pm$)-1,2-bis (3,5-dioxopiperazin-1-yl) propane (ICRF 159) has been extensively studied *in vivo* in human¹ and animal tumours^{2,3}, but its mode of action at the cellular and biochemical level has not yet been elucidated. Although it reduces the rates of DNA, RNA and protein synthesis of cells *in vitro*⁴, it has also been shown to block the cell generation cycle in the post-DNA synthesis (G_2) phase^{5,6}. Tumours whose growth is halted contain many large multinucleate cells not seen in control tumours. An attempt has therefore been made to see if the morphological changes produced by the drug in cells grown *in vitro* could provide an indication of its intracellular site of action and point towards possible mechanisms of action. The cells used were non-synchronised cultures of non-transformed hamster fibroblasts (Nil 8 cells), HeLa cells and fibroblasts derived from a transplantable fibrosarcoma in Syrian hamsters (T17 cells). They were exposed to single daily doses of ICRF 159 for 3 d.

For the first 24 h, growing cultures of Nil 8, T17 and HeLa cells in concentrations of ICRF 159 up to $20 \mu\text{g ml}^{-1}$ increased in number at the same rate as the controls. Between 24 to 48 h, however, the rate of cell increase was reduced by the drug in inverse proportion to its concentration. Between 48 to 72 h, cell numbers continued to increase in concentrations up to $10 \mu\text{g ml}^{-1}$ (Table 1). At the same time an analysis of

the distribution of cell sizes using a Coulter counter showed that Nil 8, T17 and HeLa became bigger with increasing time in increasing concentrations of ICRF 159 (Fig. 1).

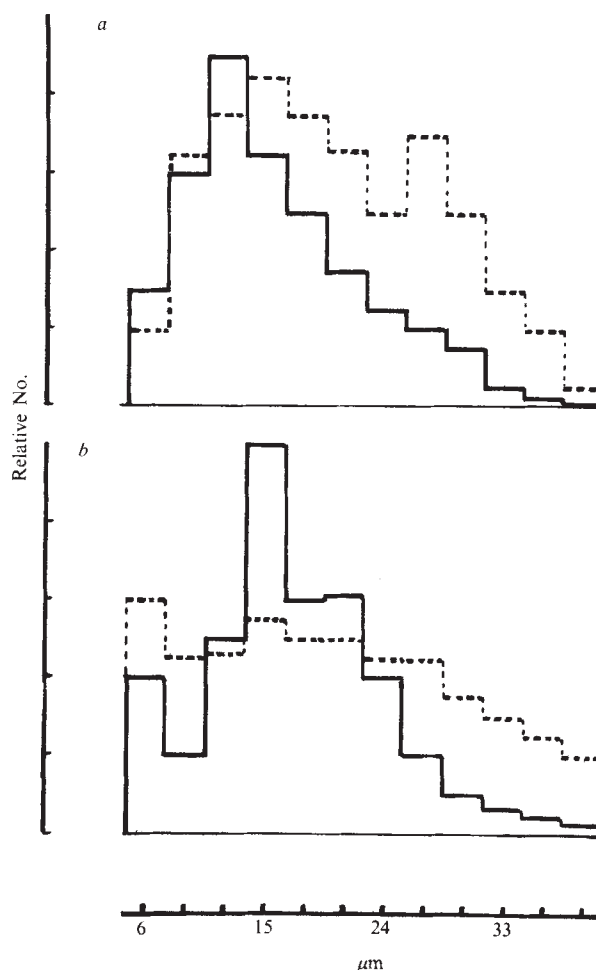


Fig. 1 Distribution of cell diameters of, a, T17 cells, b, HeLa cells after 72 h culture in either $0 \mu\text{g}$ (—) or $20 \mu\text{g}$ (---) ICRF 159 ml^{-1} . The cells were plated at 1×10^5 cells per 5 cm dish and grown in Dulbecco's modified Eagle's medium plus 10% foetal calf serum at pH 7.2. ICRF 159 was added after 90 min, 24 and 48 h after plating. The culture medium and cells removed by trypsinisation, were centrifuged, resuspended in saline and counted and sized in a Coulter counter calibrated for $3 \mu\text{m}$ diameter per window.

Table 1 The effect of ICRF 159 on three cell lines

Cell lines	Hours of culture	Numbers of cells ($\times 10^5$) per dish in medium containing ICRF 159					
		$0 \mu\text{g ml}^{-1}$	$2.5 \mu\text{g ml}^{-1}$	$5 \mu\text{g ml}^{-1}$	$10 \mu\text{g ml}^{-1}$	$20 \mu\text{g ml}^{-1}$	$40 \mu\text{g ml}^{-1}$
Nil 8	24	2.5	2.5	2.5	2.5	2.3	2.0
	48	5.5	5.0	3.0	2.7	1.7	1.0
	72	16.2	12.1	10.0	1.5	1.4	0.9
T17	24	2.5	2.5	2.5	2.3	2.0	2.0
	48	8.7	7.0	4.2	4.0	3.5	2.0
	72	21.4	12.0	5.3	2.0	1.9	1.5
HeLa	24	2.9	2.9	2.5	2.0	2.5	2.0
	48	8.0	6.2	4.5	3.5	3.0	1.5
	72	15.0	12.4	10.0	3.0	2.5	1.0

The cells were plated at 1×10^5 per 5 cm dish at 0 h, ICRF was added 90 min, 24 and 48 h later. The cells were trypsinised from the dishes and their number counted in a haemocytometer. The results are derived from duplicate experiments using three dishes per experimental point.

Morphological studies confirmed that Nil 8, T17 and HeLa cells became larger when exposed to concentrations of ICRF 159 greater than $5 \mu\text{g ml}^{-1}$ for 24 h or longer and showed that numbers of bi- or multinucleate cells appeared in the cultures. These changes were most noticeable after 72 h culture in $20 \mu\text{g ml}^{-1}$. HeLa cells differed from Nil 8 and T17 cells in that a significant proportion of dead HeLa cells were seen (dye-exclusion test) in concentrations of ICRF 159 that produced few if any dead Nil 8 or T17 cells. The dead cells were predominately small mononuclear cells. Other workers in these laboratories have shown that cultured BHK-215 cells became multinucleated when treated with ICRF 159 and by time-lapse cinematography have demonstrated that this is the result of an interference with the separation of daughter cells (T. C. Stephens and A. M. Creighton, unpublished).

Ultrastructural study, however, showed that increasing cell size was not accompanied by osmotic or degenerative changes in Nil 8 and T17 cells although a few of the HeLa cells con-

tained cytoplasmic hydropic vacuoles. Surprisingly, the large mono-, bi-, or multinucleate cells contained normal-looking cell organelles (Fig. 2). The numbers of mitochondria and ribosomes were increased in proportion to other cytoplasmic organelles, the nuclei of the large cells were often deeply folded and nucleoli were mostly present. Occasional mitotic figures were seen, the centrioles and spindle tubules were normal both in the small and large cells, but the appearance of the centriole in some cells suggested that they were in G_2 (ref. 7). This may be the point at which the dividing cells are blocked as has been previously found⁵. Although centrioles were often present in cells from control cultures, none resembled those seen in G_2 . Occasional cells in control and ICRF 159-treated cultures had both electron-dense nuclei and cytoplasmic organelles and peripheral cytoplasmic projections. They were most abundant in HeLa cells after 72 h in $20 \mu\text{g ml}^{-1}$ ICRF 159. These cells resembled apoptotic cells⁸.

Table 2 Labelling index of T17 cells after culture in ICRF 159

($\mu\text{g ml}^{-1}$)	Culture (h)	Increase in cell number ($\times$)	Labelling index
0	0 to 24	2.5	80 ± 3
	24 to 48	2.8	78 ± 3
	48 to 72	2.4	79 ± 2
	0 to 72	16.0	90 ± 3
5	0 to 24	2.5	88 ± 2
	24 to 48	1.8	70 ± 2
	48 to 72	1.1	70 ± 2
	0 to 72	5.2	85 ± 3
20	0 to 24	2.1	82 ± 2
	24 to 48	1.5	59 ± 2
	48 to 72	0.7	59 ± 2
	0 to 72	2.2	85 ± 4

The cells were plated at 1×10^5 at 0 h and ICRF 159 and $0.2 \mu\text{Ci}$ /methyl- ^3H thymidine were added 90 min, 24 and 48 h later. After trypsinisation, the cells were processed as for electronmicroscopy (Fig. 2). Sections $0.5 \mu\text{m}$ thick, were mounted on glass, dipped in NTE K5 emulsion, exposed for 1 to 7 d and developed in Kodak D19. 500 Cells were counted for each point and the results are derived from triplicate experiments.

The incorporation of methyl- ^3H thymidine in the presence of ICRF 159 was studied in T17 cells (Table 2). The labelling index of both control and ICRF 159-treated cells was similar during the first 24 h whereas the index was greater in control cells than in treated cells during the 24 to 48 and 48 to 72 h periods. Cells cultured for up to 72 h in even $20 \mu\text{g ICRF 159 ml}^{-1}$ were still capable of thymidine incorporation although the concentration was sufficient to inhibit cell division. Moreover, the intensity of nuclear labelling (grains per nucleus) was similar in cells from control and drug-treated cultures during 0 to 24, 24 to 48 and 48 to 72 h periods. The multilobed nuclei were as capable of DNA synthesis as were the nuclei of mononuclear cells. Where a binucleate cell was labelled, both nuclei had similar grain counts. A high proportion of unlabelled nuclei during the 48 to 72 or 0 to 72 h periods were either fragmented or of a bizarre shape.

As many of the cells cultured up to 72 h in ICRF 159 were viable and able to synthesise DNA, the relationship between length of exposure, drug concentration and cell numbers was further investigated. *In vivo* studies have shown that a single dose of ICRF 159, however large, has little or no effect on tumour growth⁹, whereas quite small doses repeated on three consecutive days could produce complete inhibition (K. H., and K. Burrage, unpublished). T17 cells were therefore cultured for either 0 to 24 h, 0 to 48 h or 0 to 72 h in medium that contained 2.5 to $20 \mu\text{g ICRF 159 ml}^{-1}$ and, after washing for 30 min with drug-free medium, were allowed to continue their growth in drug-free medium for the next 48 to 96 h. The increase in cell numbers 48 h after a 24 h exposure to the drug was slightly less than in control cultures (Fig. 3) whereas it was much less after a 48 h exposure. During incu-

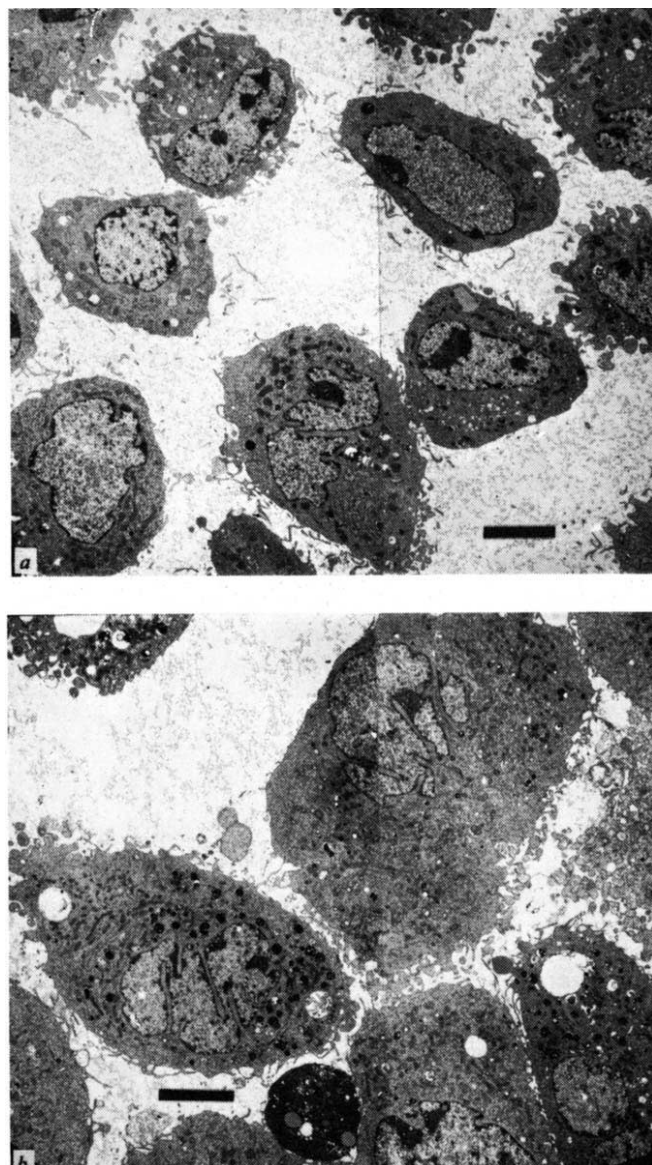


Fig. 2 Comparison of the ultrastructural morphology of T17 cells after 72 h culture in either, *a*, $0 \mu\text{g}$ or, *b*, $20 \mu\text{g ICRF 159 ml}^{-1}$. After trypsinisation, the cells were fixed with 2.5% glutaraldehyde in Sorenson's phosphate buffer, gelled in albumin-glutaraldehyde, secondarily fixed in 1% osmic acid, dehydrated and polymerised in Araldite. The ultrathin sections were stained with methanolic uranyl acetate and aqueous lead citrate. The cells treated with ICRF 159 are larger than untreated cells, have increased numbers of normal cytoplasmic organelles and show exaggerated nuclear folding. The scale is equivalent to $5 \mu\text{m}$.

bation in drug-free medium, cell numbers increased after an initial lag period that was proportional to the dose of ICRF 159 but cell numbers showed some recovery even after exposure to $20 \mu\text{g ml}^{-1}$. During the recovery phase the numbers of small mononuclear cells increased whereas the numbers of large mono- or binucleate cells remained fairly constant. Cultures exposed to $5 \mu\text{g}$ or more of ICRF 159 for 0 to 72 h before washing and reculturing in drug-free medium showed little, if any, recovery of cell numbers after 96 h further culture (Fig. 4).

Studies on erythroid maturation have shown that the cytostatic effect of ICRF 159 was seen only in dividing cells¹⁰. To confirm that no morphological changes were produced on non-dividing cells, the effect of ICRF 159 on confluent cultures of Nil 8 cells was studied as these cells show complete contact inhibition of cell division without becoming detached

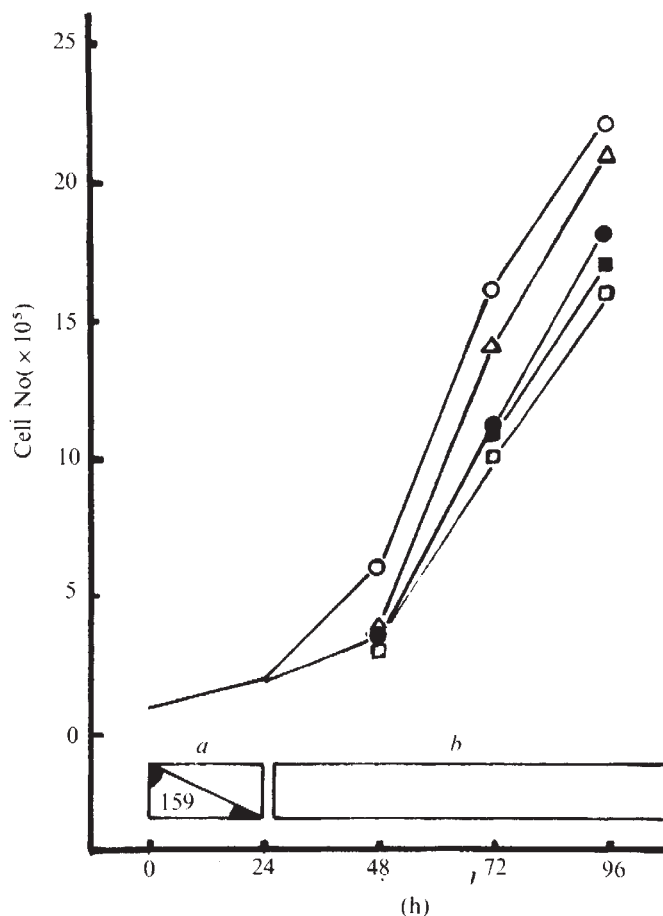


Fig. 3 Effect of 0 to 24 h culture in medium containing ICRF 159 on numbers of T17 cells. The cells were plated at 1×10^5 per dish at 0 h, ICRF 159 was added 90 min later. After 24 h culture the medium was removed and replaced with drug-free medium. The numbers of cells per dish was determined 24, 48 and 72 h later. *a*, Falling concentration of ICRF 159 due to chemical degradation in culture medium during the 24 h period; *b*, drug free medium. $\circ$, Control; $\triangle$, 2.5 μg ICRF 159 ml^{-1} ; $\bullet$, 5 μg ICRF 159 ml^{-1} ; $\blacksquare$, 10 μg ICRF 159 ml^{-1} ; $\square$, 20 μg ICRF 159 ml^{-1} .

from the base of the plastic dishes. Confluent cultures of Nil 8 cells were exposed for 0 to 72 h to concentrations of ICRF 159 of either 5 or 20 μg ml^{-1} . The cells were removed from the dishes by trypsin, their numbers counted and equal numbers plated into dishes containing drug-free medium. After a further 48 h culture, no differences were found either in cell morphology or numbers per dish between confluent cells cultured with or without ICRF 159. It was possible that the degradation products of ICRF 159 accumulating in the culture medium during 0 to 72 h were inhibitory to cell growth. It was, therefore, used as the growth medium for non-confluent Nil 8 cells. The cells increased in numbers during the next 48 h at a greater rate than did similar cells in control medium. The degradation products of the drug are therefore not inhibitory to cells growing in conditioned medium.

Other chemotherapeutic agents induce the formation of mono- and binuclear giant cells in culture. Several nitrogen mustards allow DNA synthesis to continue above the pre-mitotic level, although this treatment may result in cells blocked at either the end of S (ref. 12), S/G₂ (ref. 13) or during G₂ (ref. 14). It seems, therefore, that if nuclear DNA synthesis is permitted, but cell division is blocked at either S/G₂, G₂ or G₂/M, multinucleate giant cells will appear in the culture and their appearance is both dose and time dependent. The effects of ICRF 159 on cultured cells reported

here are compatible with an interference with the mitotic cycle during S/G₂, G₂, or G₂/M.

Several conclusions clearly emerge from the present studies. The drug only acts on cells during their generation cycle. It is without effect on resting cells. The inhibitory action is proportional to the drug concentration in the medium. The inhibition is reversible after one exposure to the drug, but cumulative if the concentration is above a threshold level and given repeatedly. It is to be expected that drug concentrations and length of exposure required to produce inhibition

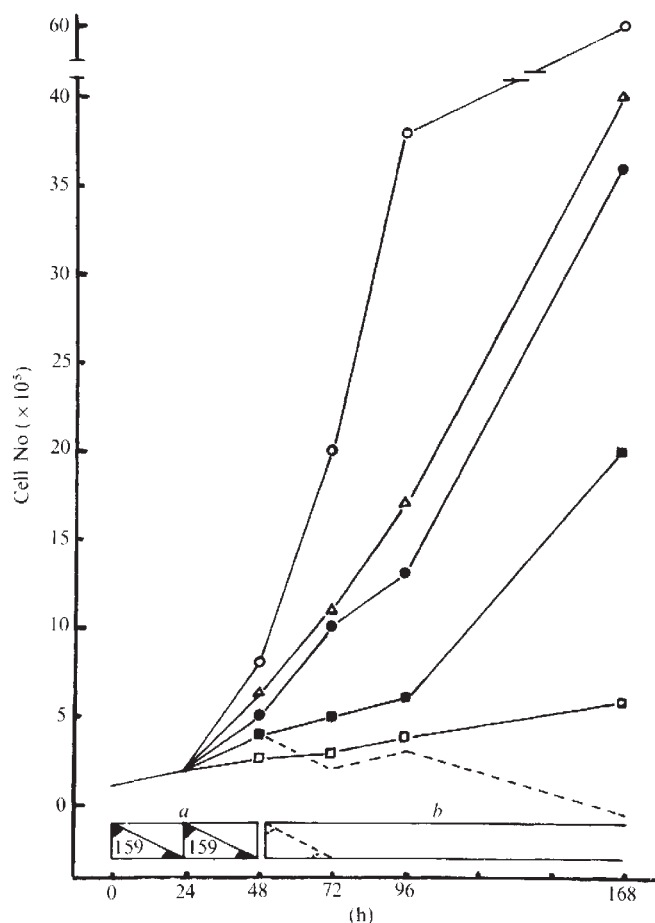


Fig. 4 Effect of 48 h culture in medium containing ICRF 159 on numbers of T17 cells. The cells were plated at 1×10^5 per dish at 0 h, ICRF 159 was added 90 min and 24 h later. After 48 h culture the medium was removed and replaced with drug-free medium. The numbers of cells per dish was determined 24, 48 and 72 h later. (---), additional 10 μg ICRF 159 added at 48 h and the medium changed at 72 h. *a*, Falling concentration of ICRF 159 due to chemical degradation in culture medium during the 24 h period; *b*, drug-free medium. $\circ$, Control; $\triangle$, 2.5 μg ICRF 159 ml^{-1} ; $\bullet$, 5 μg ICRF 159 ml^{-1} ; $\blacksquare$, 10 μg ICRF 159 ml^{-1} ; $\square$, 20 μg ICRF 159 ml^{-1} .

of cell division will vary with cell types. Because of the remarkable lack of structural damage which accompanies these very subtle, but precise insults to the cell division mechanism, however, and because of doses of the drug which ultimately inhibit division, but permit the cells to go normally through DNA synthesis, the present studies on non-synchronised cells have not been able to pin-point the intracellular site or the biochemical mode of action of the drug. They are, however, in excellent agreement with earlier *in vitro* work which indicated that the block of cell division occurred at the end of G₂ or beginning of mitosis and with *in vivo* findings that single large doses are without effect and that at least 3 consecutive doses are required for antitumour activity. It needs to be stressed, however, that *in vivo* tumour systems appear to be much more sensitive to the action of ICRF 159 than the present *in vitro* studies would suggest.

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inoculation of cells into newborn rats where transformed cells produce progressively growing tumours.

The cumulative effects of initiation with benzo(a)pyrene and promotion with phorbol ester on the incidence of morphologically transformed colonies that arose from cells cloned in liquid medium are summarised in Table 1. These results show that, until the twenty-first passage, neither initiation alone nor initiation followed by promotion for six cell passages, gave a marked increase in morphological transformation. Subsequently the incidence of colonies showing morphological transformation increased sharply in both groups of treated cells but those cells treated with both initiator and promoter showed a higher efficiency of transformation. The incidence of spontaneous transformation in the untreated cells was only significantly increased after forty passages (Table 1).

Table 1 Transformation of Cells Treated with Benzo(a)pyrene and Phorbol Ester and Cloned in Liquid Medium

Pas- sage No.	Treatment *		Transformation assay			
	Initiation with benzo(a)- pyrene	Promo- tion with phorbol ester	No. of colonies	Plating efficiency (%)	No. of trans- formed colonies	Efficiency of trans- formation (%)
8	1	0	397	39.7	10	2.5
	0	0	312	31.2	4	1.4
10	1	0	258	25.8	6	2.3
	0	0	174	17.4	3	1.7
21	1	6	384	31.2	5	1.3
	1	0	288	28.8	7	2.4
	0	0	282	28.2	0	0
34	1	19	123	12.3	41	33.0
	1	0	122	12.2	25	20.5
	0	0	219	21.9	3	1.4
40	1	25	388	24.3	235	60.6
	1	0	504	31.5	214	42.5
	0	0	479	24.0	55	11.5

Embryonic Wistar rat fibroblasts were obtained from 14-d-old embryos that were removed under sterile conditions, washed twice in phosphate-buffered saline (PBS) and cut into fragments which were stirred for 5 min in PBS and then submitted to the repeated action of 0.25% trypsin at 37° C. The supernatant from the first 20 min digestion was discarded, and those from the next three digestions, each of 10 min, were combined, filtered through sterile gauze and centrifuged. The pellet was resuspended in Dulbecco's growth medium supplemented with 10% foetal calf serum and this cell suspension was used to prepare primary cultures. After counting and diluting, 10⁴ cells were seeded into 10 cm Petri dishes (Falcon Plastics) that contained 10 ml medium, and the dishes were incubated at 37° C in an atmosphere of 10% CO₂. The cells were subsequently subcultured every 5 d. Following the third subculture the Petri dishes were divided into groups and benzo(a)pyrene (Schuchardt) added, as an acetone solution, to the medium in one group of dishes to give a benzo(a)-pyrene concentration of 1 µg ml⁻¹, and an acetone concentration of 0.5%. Another group of dishes remained untreated. After 2 h the cells in both groups were subcultured. After a further thirteen passages, half the dishes of treated cells were continuously exposed to phorbol ester (12-O-tetradecanoyl-phorbol-13-acetate, Schuchardt) added as an acetone solution to give a final concentration of 0.01 µg phorbol ester per ml of medium, the ester being added at each medium change. Estimations of plating efficiency and morphological transformation were made at intervals on 200 cells from each group that were cloned in the supplemented growth medium and which, after 9 d, were then fixed in methanol and stained with May-Grunwald and Giemsa.

* Number of passages during which benzo(a)pyrene or phorbol ester were present in the cell medium.

When cells were examined at intervals during the experiments for their ability to form multicellular colonies in semi-solid agar medium, the results given in Table 2 were obtained. These show that although some cells treated with benzo(a)-pyrene alone developed the ability to form colonies, normally of only three to five cells, those subsequently grown in medium that contained phorbol ester often developed into dense foci that consisted of more than twenty cells. The untreated cells did not acquire the ability to form colonies in these experiments before the fortieth passage and remained isolated as single cells in suspension in the agar medium.

Although both morphological transformation and colony

Two-stage Malignant Transformation of Rat Fibroblasts in Tissue Culture

MOST of the *in vitro* systems that have been developed to study the mechanisms of chemical carcinogenesis use rodent fibroblasts grown in culture¹⁻³. Since they are rapid and permit the control of many parameters, these *in vitro* malignant transformation techniques offer advantages over the *in vivo* procedures previously employed.

Co-carcinogenesis, which occurs in two distinct stages, those of initiation and promotion⁴⁻⁷, has been demonstrated in skin⁸⁻⁹, in the forestomach of the mouse¹⁰ and in other tissues¹¹. 7,12-Dimethylbenzo(a)anthracene¹², benzo(a)pyrene¹³ and urethane¹⁴ have been the most commonly used initiating agents while croton oil¹⁵ and phorbol ester¹⁶⁻¹⁷, an active constituent of this plant extract, have frequently been employed as promoting substances. Analysis of the cellular changes that occur in co-carcinogenesis *in vivo*, however, is not easy; for example, metabolism that leads to the inactivation and excretion of the carcinogen and variations in the turnover of cells in the treated tissue are two of the factors that complicate quantitative investigations of co-carcinogenesis in animals. Here we report results which show that malignant transformation of rat fibroblasts grown *in vitro* can proceed in two stages, those of initiation and promotion.

The experiments were carried out with embryonic fibroblasts from Wistar rats, grown in culture and serially passaged every 5 d¹⁸. Cell cultures were treated with benzo(a)pyrene or with phorbol ester or with both compounds which were added to the medium as acetone solutions; other cell cultures remained untreated. Tests for cell transformation were made on the subcultures at intervals as shown in Tables 1-3. The three criteria of *in vitro* malignant transformation that were used have been described previously¹⁸ and consist of (1) the cloning of cells in liquid medium using a feeder layer composed of irradiated cells, transformation being recognised by the abnormal morphological arrangement of the cells in the colony^{3,19}, (2) the cloning of cells in agar suspension; transformed cells have the ability to develop into three-dimensional colonies under these conditions²⁰ and (3) the subcutaneous

formation in semisolid medium are accepted as being indicative of malignant transformation in cultured cells, only the induction of tumours in experimental animals by such cells is generally regarded as proof. In the experiments described here, progressively growing tumours regularly developed only in those

Table 2 Colony Formation in Semisolid Agarose Medium by Cells Treated with Benzo(a)pyrene and Phorbol Ester

Passage No.	Treatment *		Colony formation in agar medium
	Initiation with Benzo(a)pyrene	Promotion with phorbol ester	
29	1	14	++
	1	0	+
	0	0	—
34	1	19	+++
	1	0	+
	0	0	—
40	1	25	+++
	1	0	++
	0	0	±

Rat fibroblasts obtained, cultured and treated as described in the legend to Table 1 were examined for colony forming ability in semisolid medium²⁰. Cells (1×10^4) were plated in 0.3% agarose medium and incubated at 37° C in an atmosphere of 5% CO₂. After 10 d unstained cultures were scored for colony formation. Symbols used above are —, single cells; +, colonies of three to five cells; ++, numerous colonies of ten to twenty cells; +++, numerous colonies of thirty to thirty-five cells.

* Number of passages during which benzo(a)pyrene or phorbol ester were present in the cell medium.

animals injected with cells that had been initiated with benzo(a)pyrene and then promoted with phorbol ester (Table 3); these tumours were all palpably detectable 3 weeks after injection of 2×10^6 cells and were transplantable. Cells that had been treated with initiator alone occasionally gave rise to a more slowly growing tumour. On histological examination, the tumours were identified as fibrosarcomas^{3,18,19} in which sarcomatous cells in close contact showed a fusiform orientation and atypical nuclei. No tumours have been detected to date in any of the animals injected with untreated cells (Table 3). In other control experiments, uninitiated rat fibroblasts were treated continuously with phorbol ester. Although, after twenty-nine passages in medium containing the promoter, these cells gave rise to a few colonies of up to twenty cells in semisolid agarose medium, this colony forming ability was not apparent before the twenty-fifth passage; when reinjected into animals, cells treated with phorbol ester alone did not give rise to any tumours.

Taken together these observations show that malignant transformation of rat embryonic fibroblasts has been obtained in tissue culture by the sequential action of benzo(a)pyrene and phorbol ester. In addition to morphological alterations, this malignant transformation was characterised both by the ability of transformed cells to form colonies in agar medium and by the formation of tumours when such cells were reinjected into animals. Whereas the latter criterion provides the best evidence for malignant transformation, the apparent ability of chemically transformed cells to form colonies in semisolid medium merits further study. Chemically transformed rat fibroblasts resemble hamster kidney cells transformed with polyoma virus in this respect²⁰ and the use of this technique could offer an alternative quantitative method for assessing malignant transformation *in vitro*. In addition, it may be possible to use the growth of chemically treated cells cloned in agar medium in order to investigate the stage of malignant transformation that has been reached since both the frequency with which colonies form and their dimensions seem to vary with the treatment given¹⁸. On the other hand, this technique does not enable a study of the morphological aspects of the growth of transformed cells since, in agar medium, they adopt a spherical form.

The two-stage induction of malignant transformation *in vitro*

Table 3 Tumour Formation *in vivo* by Cells Treated *in vitro* with Benzo(a)pyrene and Phorbol Ester

Passage No.	<i>In vitro</i> treatment *		<i>In vivo</i> tumour formation		
	Initiation with benzo(a)-pyrene	Promotion with phorbol ester	No. of cells injected $\times 10^6$	No. of animals with tumours	Time for appearance of tumours (weeks)
22	1	7	2	0/13	
	1	0	2	0/10	
	0	0	2	0/10	
28	1	13	2	0/12	
	1	0	2	0/12	
	0	0	2	0/10	
37	1	22	2	10/10	3
	1	0	2	1/10	4
	0	0	2	0/10	
41	1	26	1	11/11	3
	1	0	1	0/11	
	0	0	1	0/11	

Cultured rat fibroblasts ($1-2 \times 10^6$) that had been treated as described in the legend to Table 1, were injected, as a suspension in culture medium (0.05 ml), subcutaneously into the cranial region of 2-d-old Wistar rats which were subsequently examined at weekly intervals for palpable tumour formation.

* Number of passages during which benzo(a)pyrene or phorbol ester were present in the cell medium.

closely parallels the co-carcinogenesis that has been described *in vivo*¹³ for, in both situations, a single treatment with benzo(a)pyrene, for example, initiates cells which can subsequently be promoted to overt malignancy by continuous treatment with phorbol ester. The demonstration that co-carcinogenesis can occur in cells in culture may facilitate studies relevant to human disease. It is accepted that a variety of initiating and promoting agents are present in tobacco smoke²¹ and in the urban atmosphere²². These undoubtedly contribute to the induction of cancer of the respiratory tract in man which probably represents a widespread example of chemical co-carcinogenesis.

We thank Dr P. L. Grover for advice.

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Received October 26; revised November 29, 1973.

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Prostaglandins as Mediators of Reactive Hyperaemia in Kidney

SEVERAL mechanisms have been proposed to account for the reactive hyperaemia which follows arterial occlusion in the kidney, including the intrarenal production of a vasodilator substance^{1,2}. Recent work shows that the local generation of a prostaglandin can cause vasodilatation in the kidney^{3,4} and can contribute to autoregulation of renal blood flow^{5,6}. We have therefore used a prostaglandin synthetase inhibitor, indomethacin⁷, to test whether the reactive hyperaemia which follows release of renal artery occlusion is mediated by a prostaglandin mechanism.

Ten mongrel dogs of either sex weighing 9.5–28 kg, were anaesthetised with pentobarbitone sodium (30 mg kg⁻¹) and ventilated with a respiratory pump. Systemic blood pressure was measured with a Statham pressure transducer from a femoral or brachial artery. The left kidney was exposed by transabdominal incision and the renal artery was isolated to a length of 4–5 cm. Care was taken to minimise manipulation of the kidney and of the renal nerves. Renal blood flow was measured with an electromagnetic flowmeter (Biotronix, Model BL-61 0). The ureters were cannulated and urine output was measured with drop counters. Renal blood flow, urine output and systemic blood pressure were recorded continuously on a Beckman Type S-II Dynograph.

The renal artery was occluded with a clamp placed distally to the flowmeter probe. Occlusion was maintained for 3 min and then released. After reproducible effects had been obtained, an intravenous infusion of 0.9% NaCl was given in a dose of about 30 ml kg⁻¹. The fluid volume of the animal was then maintained constant by saline injected from an automatic syringe which replaced each ml of urine excreted with an equivalent amount of saline. Occlusion of the renal artery was then repeated before and after indomethacin (1–5 mg kg⁻¹ intravenously). In three experiments sodium content was measured in the urine samples collected in 10–15 min intervals before and after indomethacin administration.

The rate of blood flow through the kidney varied from 100–400 ml min⁻¹ in different experiments. The patterns of flow changes after the release of arterial occlusion differed between animals. There was always an initial brief overshoot of flow which was followed either by a gradually reducing phase of increased flow or by a transient decrease before the flow returned to the pre-occlusion value. After saline load, the brief overshoot was higher and the phase of increased flow was potentiated and prolonged. The transient decrease in flow which followed release of occlusion in some experiments was also abolished. Indomethacin administration induced a decrease in resting renal blood flow, urine output and sodium excretion and a reduction of the reactive hyperaemia which followed release of occlusion. The reduction in resting flow ranged from 5% to 24% of the initial value. The average decrease following administration of 1, 2 or 5 mg kg⁻¹ of indomethacin was 9%, 13% and 18% respectively. Urine output decreased in all animals and the sodium content of the urine was reduced from 7% to 25% within 1 h after indomethacin administration.

The reactive hyperaemia following release of renal arterial occlusion was decreased or abolished after indomethacin (Fig. 1). Although the initial brief overshoot was not much reduced, the slower phase of increased flow was either reduced (five experiments) or completely abolished (four experiments). In one animal, which interestingly had a raised blood pressure (175/100 mm Hg), indomethacin did not change the reactive hyperaemia.

In the anaesthetised dog, there is a continuous output of a prostaglandin into the renal vein^{6,8}. The output is increased by various manoeuvres, including ischaemia⁹ and reduction in perfusion pressure⁶ and the prostaglandin has been identified as mainly E₂ (ref. 9), which is a potent renal vasodilator.

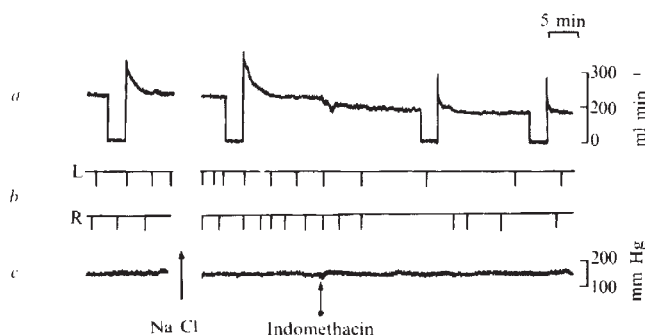


Fig. 1 Effects of intravenous saline and indomethacin on the post-occlusive hyperaemia in the kidney. *a*, Renal blood flow of the left kidney; *b*, urine output (ml) from each kidney; and, *c*, blood pressure of a 9 kg female dog. Each period of occlusion (3 min) was followed by a hyperaemia, the magnitude of which was increased by intravenous saline (30 ml kg⁻¹). After indomethacin (2 mg kg⁻¹ intravenously) resting renal blood flow decreased, as did urine flow. All but the fast phase of post-occlusive hyperaemia was abolished.

Indomethacin reduces renal blood flow and abolishes the prostaglandin output^{3,6}. Our experiments have now shown that the reactive hyperaemia which follows renal artery occlusion is also reduced or abolished by indomethacin. Thus, the most likely interpretation of our results is that the reactive hyperaemia is at least partially due to a prostaglandin mechanism. It could be that during cessation of blood flow, the basal output of prostaglandin continues within the kidney, or that the ischaemia which is produced actually increases the output. In either situation, the increased concentration of prostaglandin would cause increased vasodilatation, which would express itself by the reactive hyperaemia when blood flow is re-established. As the increased concentration is washed away by the blood, so the vessels would return to their previous tone.

The increase in the reactive hyperaemia which followed saline infusion into the dog also supports this mechanism, for prostaglandins are natriuretic¹⁰ and saline load increases renal prostaglandin output¹¹. Thus, an increased prostaglandin production in the kidney may contribute to the increase in urine flow which follows the saline load.

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book reviews

Science of science

Science Observed: Science as a Social and Intellectual Activity. By F. R. Jevons. Pp. 186. (Allen and Unwin: London, August 1973.) £3.75 boards; £2.25 paper.

THE science of science has developed rapidly into a somewhat subsidiary but on the whole almost respectable field of scholarly endeavour. The lack of any comprehensive and generally accepted model of the structure and dynamics of scientific and technological activity, however, cripples much of the work undertaken in this area. It may well be that a "unified field theory" will never really emerge in this particular branch of the social sciences any more than in others. In the meantime, students of science are understandably eager to grasp at the small number of more general hypotheses which have emerged and which seem to bring some element of order into a chaotic universe—often, one is tempted to say, at the expense of reality. Thus, an overview and assessment of progress in the field calls less for synthesis and compilation of a well established body of knowledge than for an explicit and critical examination of the extensive application of a few widely accepted assumptions which may, however, be justified only within very specific and limited conditions.

Professor Jevons has succeeded in providing—in a wonderfully clear, concise and precise manner—with just such an assessment. No doubt one could point to some of the gaps in his presentation, which does not deal directly with the science policy structure that has emerged in recent decades to become an essential component of modern science, or with the controversies on the so-called differences between hard and soft sciences. But *Science Observed* accomplishes its essential objective in putting into perspective some of the basic concepts of science studies in the past decade.

To begin with, the author establishes the new social dimension which calls for more thorough understanding of the scientific universe and the mores of its inhabitants: knowledge is a prerequisite of planning and, however awkward, the planning of science is unavoidable when the waging of war, the limitation of environmental pollution, and the creation of wealth have induced society to enlist the service of a rapidly growing

body of researchers. Thus, Weinberg's internal and external criteria are, in many ways, a challenge to provide planners with knowledge of science's internal mechanisms and their relations with society.

The scientific study of science is generally expected to produce objective and quantitative knowledge. Numbers of scientific journals, of abstracts, of papers or of citations are some of the indicators most widely used. But these indicators are not straightforward reflections of quality and progress in science, and are influenced by many extra-scientific features of the research profession and the academic marketplace. Thus, "numerology is interesting but should be used only with the utmost circumspection" since "in particular cases it could be wildly misleading" (page 45). The case would even be strengthened if the author had explored some of the abuses and inaccuracies of research and development statistics.

Unfortunately, as the author points out, the disappointments of quantification are not compensated by reliable qualitative analysis. What is scientific method? Neither the inductive or the hypothetico-deductive theories really account for "inspired guesses" and "intuitive hunches" which so often seem to underlie scientific creativity. In a historical perspective, T. S. Kuhn's exploration of the structure of scientific revolutions may provide an ideal pattern of evolution of, at least, some disciplines. It remains that his attractive distinction between "normal" and "revolutionary" research is not, in practice, as clear cut as one might wish: "it is difficult not to be convinced by direct inspection of science that contributions form a continuous spectrum" (page 69).

Lacking an acceptable formulation of the laws of the scientific universe, can one at least determine what moves its actors? The striving for recognition by fellow scientists, which has often been presented as the key motivation to scientific work seems only to apply to limited numbers and circumstances, leaving aside such modern developments as teamwork, expansion of research activities in non-academic institutions and increasingly complex career patterns.

The opacity of the internal workings of science is matched by that of the relations between science and society.

For example, in spite of many decades of scrutiny, the processes governing the contributions of science to economic growth remain uncharted. Only the complexity of the factors involved become more and more apparent.

Professor Jevon's new book, with its careful exposition and appraisal of the meagre results accumulated to date (an anthology of fundamental writings on the subject is provided in the appendices) should, it is hoped, inspire new, unprejudiced and more ambitious efforts to clarify the complex dynamics of the internal structures and external links of the system of science. It should also serve to caution educators and policy makers against short cuts and rash approximations in dealing with a system that is still not understood. If these results are achieved, the concluding note of optimism on the future of technology may turn out to be not as unrealistic as it may seem today.

GEORGES FERNÉ

High energy physics

Particle-interaction Physics at High Energies. By S. J. Lindenbaum. Pp. xiii+512. (An International Series of Monographs on Physics.) (Clarendon: Oxford; Oxford University: London, October 1973.) £15.00.

THE author of this scholarly work is an internationally recognised experimental physicist and expert, having made his own original contributions at the Brookhaven National Laboratory in this research field on several topics. The book starts at the level of the postgraduate student in high energy physics, and should appeal primarily to research workers in the field. The style is not particularly pedagogic, nor does one set out from first principles. Instead the book traces the development and reviews progress made upon particular topics. The first three chapters deal briefly with introductory matter about pions and meson field theory. The following three chapters contain considerable detail on pion-nucleon scattering, dispersion relations and models of pion production. The next four chapters comprise concise summaries of topics concerning kaon and hyperon physics, particle classifications, and electron scattering. The penultimate chapter is concerned with weak interactions, and the

final one is devoted to a substantial review of the field of total cross sections and elastic scattering at very high energies.

From these contents, it is clear that the author has leaned towards the topics with which he has been most directly involved, particularly the physics of hadronic interactions.

The omission of the considerable work done in the last decade to study the hadronic interactions of photons, underlines the dilemma in which any author tackling this subject is placed, since it is impossible to cover all aspects and be up to date at the same time. But the author has taken on, quite successfully, a prodigious task and can be congratulated on seeing this through to its end. The shorter chapters come over well as concise, yet essentially comprehensive, statements of the facts, while in the main parts of the book, one is left in no doubt as to the author's comprehensive and detailed knowledge of his topics, and of the considerable experimental and theoretical activity in the field over the past twenty years. The reference lists are extensive, and the well-known "Tables of Particle Properties", originating from the "Particle Data Group", round off the volume.

The production standards of the book are of the highest quality, and typographical errors, though present, are rare. Appropriate libraries should be encouraged to purchase the book.

W. GALBRAITH

Serious black holes

Black Holes: Les Houches 1972. Edited by C. DeWitt and B. S. DeWitt. Pp. xii + 552. (Lectures delivered at Summer School of Theoretical Physics of the University of Grenoble.) (Gordon and Breach: New York, London and Paris, 1973.) £13.50.

THIS book has already established itself as an essential work for those interested in black holes. The publication is timely because after some years of theoretical studies evidence is now mounting that black holes may have been observed indirectly in some X-ray sources. Both theoretical and observational aspects are treated in these lectures.

The book begins with Hawking's lectures on the "Event Horizon"—the "one way membrane" which separates the exterior of a black hole from the hidden interior. In them he establishes the general properties of the event horizon using the topological methods which were developed by Penrose, Geroch and himself to prove the singularity theorems which show that

according to general relativity the outcome of catastrophic gravitational collapse will be a space time singularity. By assuming that these singularities will lie hidden inside an event horizon ("Cosmic Censorship Hypothesis") he shows that the event horizon must be a null surface any connected component of which may not bifurcate and whose area must not decrease with time. He goes on to establish that a stationary black hole must be static or axisymmetric and have spherical topology. Then he applies these ideas to the flow of energy and angular momentum into black holes and to time symmetric black holes.

The flow of energy and angular momentum down a hole is taken up by Carter, who restricts most of his lectures to asymmetric, stationary black holes and gives an account of the thermodynamics of a system of matter and a black hole. Quite apart from its relevance to black holes this is an excellent account of general relativistic thermodynamics. After discussing some important properties of the Kerr solution he goes on to show that the most likely final state of an isolated black hole is that of a Kerr black hole.

Both Carter and Bardeen give a great deal of space to the Kerr metric which must now rank with the Friedman expanding universe metric as the most important exact solutions of Einstein's equations we have. Bardeen provides much detailed material on the geodesics in this geometry and then proceeds to a study of rapidly rotating bodies in general relativity culminating in a fascinating study of self gravitating disks which is of especial importance because they are the only ones so far showing how a rapidly rotating black hole may form.

After reading so much theory the reader is then encouraged to proceed further by considering the data assembled by Gursky on the X-ray observations. In this chapter Gursky indicates some of the observational techniques and their pitfalls and argues that only accretion into compact objects can explain the data. He discusses particular sources in detail—notably Cygnus X1 which may contain a black hole and Hercules X1 which probably contains a neutron star.

From Gursky's lectures it emerges that the most interesting X-ray sources from the point of view of black holes are those in which a bright young star is orbiting an unseen companion in a close binary system. The energy of the X rays is held to arise from the accretion of matter coming from the bright primary star which is spilling over its Roche lobe. Because this matter possesses angular momentum it cannot fall directly in but forms a disc around the

companion and angular momentum and energy are transferred outwards by some viscous mechanism. In their lectures Novikov and Thorne treat this transfer of angular momentum with characteristic clarity avoiding the errors of some earlier workers. Because the precise mechanism of viscosity is as yet not understood—turbulence and magnetic fields playing a major role—they provide a rather general analysis of accreting discs leaving plenty of room for viscosity parameters to be plugged in later. They also build some detailed models however, and give a useful summary of the astrophysical principles behind the calculation of the various emission processes.

In the final set of lectures Ruffini discusses a variety of important topics amongst which stand out an attempt to determine the maximum possible mass of a neutron star on rather general physical grounds and an account of why such a maximum exists at all. This is obviously of especial importance when discussing actual binary systems. He also gives a number of calculations of the gravitational waves emitted by particles falling down a hole, though some of the calculations on charged black holes should be treated with care because the coupling between electromagnetic and gravitational perturbations seems to have been ignored. This section contains much of interest but the format (short introductions followed by photo-reproductions of reprints and preprints) does not make for easy reading or compact presentations.

To finish: this is an important book which everyone seriously interested in black holes should read; I hope for the sake of their pockets that a paperback edition will be produced soon.

G. W. GIBBONS

Methods for metals

Physical Metallurgy: Techniques and Applications. By K. W. Andrews. Vol. 1 pp. 349; vol. 2 pp. 347. (Allen and Unwin: Hemel Hempstead, August 1973.) Vol. 1 £7.95; vol. 2 £8.20.

Books of this type are usually compilations by various specialists whose contributions are under the control, to a greater or lesser degree, of an editor. The present book has the great advantage of having been written by one author of wide experience who has brought to the task an impressive expertise and has imposed on the book a most desirable uniformity of treatment. It could be argued that several of the topics, such as electron microscopy and crystallography, have been adequately dealt with

in specialist books, but there is much to be said for a concise summary of the principles of a technique together with some carefully selected examples of its use. Dr Andrews is not content simply to describe a technique, but goes to considerable lengths to provide compact quantitative treatments of the various physical principles involved, in a way which will enlighten the serious student, and refresh the memories of established scientists who may have long taken the underlying principles for granted.

The first volume is rather dominated by the subject of X-ray and electron diffraction and associated techniques, which together with an introductory outline of crystallographic principles takes up two thirds of the volume. This perhaps reflects the author's own research interests, but it is appropriate that methods which have been so effective in physical metallurgy should receive substantial emphasis. It does however mean that more recently developed techniques such as field emission and field ion microscopy receive much less attention than they deserve. Other subjects dealt with in this volume include thermal methods and solidification techniques. The former deserve a place in any book of this type, for although they reach back almost to the genesis of the subject, in their modern forms they are still extremely valuable methods of investigation. The chapter on solidification techniques is rather brief and apart from a useful treatment on zone refining does little to stress the importance of this stage in alloy preparation.

The first part of Volume 2 deals with both optical and electron microscopy. It is refreshing to see that the rapidly growing subject of quantitative metallography receives due attention, while there is a fifty-page review of electron microscopy which provides clear simple treatments of image formation and the basic contrast effects. The latter half of this volume is likely to be particularly useful, as it deals with several topics about which rather less has been written recently. In particular the chapter on the determination of internal stresses is an excellent review which again reflects a special interest of the author, who has taken pains to spell out the underlying principles of stresses on macro and micro scales, and also around dislocations. The other chapters in this volume are concerned with electrical and magnetic methods, and with internal friction techniques. The latter subject has not received much attention recently, so a compact review of this type is likely to prove most valuable.

In summary these two volumes provide a most useful survey of a number of physical techniques important in metallurgical research. The able combination of basic theory with practical

application of the techniques should appeal to a wide spectrum of readers.

R. W. K. HONEYCOMBE

Phoenix palaeontology

Evolutionary Paleocology of the Marine Biosphere. By J. W. Valentine. Pp. xv + 511. (Prentice-Hall: Englewood Cliffs, NJ, October 1973.) \$6.95.

THE past few years have witnessed striking advances in the application of modern concepts of ecology, evolutionary biology and plate tectonics to achieve an improved understanding of the fossil record, and it is appropriate that the first comprehensive review of this revitalised palaeontology should be written by one of the leading workers in this sphere. In the early part of his book Valentine gives a useful survey of evolutionary and ecological theory and of the principal physical parameters of the marine environment. He goes on in the more substantial part of the book to treat marine animals in terms of a hierarchical system. Successive chapters deal with the mode of life and functional morphology of individual organisms, populations, communities, faunal provinces and the marine biosphere, ending with general interpretation of the evolutionary history of this biosphere from the Precambrian to the present. Each level in the hierarchy can be viewed as a sort of 'black box', with its own intrinsic patterns of organisation which can often be studied without reference to lower or higher levels. Thus when dealing with patterns of community organisation one does not necessarily have to be preoccupied unduly with the relationship of form and function for particular phenotypes, and so on.

One of the most impressive features of this fine book is the overall conceptual framework created, which breaks away refreshingly from the somewhat stultifying preoccupation with particular taxa, with no particular scientific goal in mind, which has characterised so much palaeontology in the past. Another is the erection of a series of testable models. It is not that detail is ignored, far from it, but it is subsumed into a more general system so that one can evaluate more readily the relevance of making certain types of observation as a means of testing particular hypotheses.

Valentine's own research has been mainly concerned with the higher organisational levels and his important views on such matters as the environmental control on provinciality, diversity changes, mass extinctions and the evolution of basic morphological structures through geological time are nat-

urally given a good airing. The presentation of these views is, however, not dogmatic and one does not have to agree with all he says to accept that he is laying emphasis on the sorts of problems to which the palaeontological community should address itself increasingly in the future. The writing style is generally clear and where new jargon is introduced it is well defined. The production is generally good, and illustrations abundant, though the number of typographical errors is large. The book can be used with profit in advanced undergraduate courses and deserves to be read by all who want to learn more about the new palaeontology.

A. HALLAM

Biological names

Biological Nomenclature. By Charles Jeffrey. Pp. ix + 69. (Arnold: London, October, 1973.) £2 boards; £1 paper.

"WHAT! Is it possible? Not to know the principles of nomenclature? Of what can your teachers have been thinking?"

Older biologists should no longer need thus to paraphrase Rob Roy. Dr Jeffrey's book shows clearly how far there is a simple common basis to the various international codes of biological nomenclature, and how they differ in expression.

Dr Jeffrey is a botanist. A zoologist may find points to criticise, of which I mention two. First, it is not true that publication is the process of making a zoological name available (page 64) (see chapter 4 of the zoological code). Second, the notion of the "nominal taxon" is misrepresented. It is not enough to say (page 63) that it is the taxon that bears a given name: it is the taxon, as objectively defined by its type, to which a given name applies. Botanists speak of "the type of a name", which is to imply, in cases of homonymy, that one concept can have two or more different objective bases, which is a nonsense. The name is not the same thing as the concept it denotes. To Dr Jeffrey "The type of a name . . . has no significance for classification" (page 18). To a zoologist, the nominal taxon is the concept that is common to all notions of the taxon. As such, it provides the essential link between classification and nomenclature and is a notion of great practical value.

Nevertheless, biologists at all levels of experience should study this book. Dr Jeffrey and the Systematics Association have rendered a major service to all biologists whose work calls for some understanding of the basic principles of nomenclature.

R. V. MELVILLE

obituary

D. M. S. Watson

PROFESSOR D. M. S. WATSON, FRS, who died on July 23, aged 87, was trained at the University of Manchester as a chemist and geologist, but started research in palaeontology. His first palaeobotanical publication appeared when he was twenty and the second (jointly with Marie C. Stopes) was published by the Royal Society only a year later. His early papers include descriptions of a fossil king-crab and the loops of a brachiopod, and within five years of entering vertebrate palaeontology he had published on fossil fish, amphibians, rhynchosaurs, plesiosaurs, crocodiles, chelonians and mammal-like reptiles.

He developed many of these interests in later years, and in particular began to ask two questions: how did it work and why did it change? By so doing he joined Abel as one of the pioneers of palaeobiological thought, and became a leader in his field by the age of 30. Much of Watson's work was based on his own collecting in South Africa and he wrote several papers on Karroo stratigraphy. He was soon recognised as an authority on the mammal-like

reptiles, and he began to build up his experience of labyrinthodont amphibians and eotylosaurs, while exploring (jointly with Hankin) the flight of pterodactyls.

Watson was at his greatest in the period between the two World Wars, and his papers on the structure, evolution and origin of the Amphibia stand out as classics. He was appointed to the Jodrell Chair of Zoology and Comparative Anatomy at University College, London in 1921, elected Fellow of the Royal Society in 1922, gave their Croonian Lecture in 1924 and the Romanes Lecture in 1928.

D. M. S. was particularly interested in missing links in the evolutionary record because they held promise of an opportunity to study the functional aspects of transition from one mode of life to another. His quest to fill the structural gap between jawless and jawed fish led to his recognition of the apheothoid grade in acanthodians and, although his interpretation has been doubted, his thorough study of this difficult material remains a masterpiece. Other topics which he explored include the evolution of the mammalian ear,

the primitive tetrapod limb, the structure of the monotreme skull and fossil coelacanths. For many years Watson was aided in his work by Miss Joyce Townend whose excellent illustrations grace his publications.

By present standards Watson's mechanical preparation of fossils was relatively crude, but his training in geology ensured an ability to visualise three dimensional structure from a series of broken surfaces, and he could break a rock in just the right place to confirm his expectations. He was not a writer of textbooks and communicated best through his lectures, during which he radiated enthusiasm. The spirit of these is captured in *Paleontology and Modern Biology*, which contains the Silliman Memorial Lectures, essentially as they were delivered at Yale in 1937. Another fine performance was his lecture on *Pterodactyls*, which he gave on several occasions but never published.

On the personal side, D. M. S. sometimes seemed slightly reserved (he had a charmingly gentle relationship with his wife) but he always found time to listen to his research students and he was tolerant of their inexperience.

Announcements

Appointments

Major-General Sir Leonard Atkinson, KBE, has been elected the new chairman of the Council of Engineering Institutions.

Awards

The 1973 International Health Foundation Award for papers on *Diagnostic X-ray exposure and the human embryo* will go to Dr Willem Herstel of the Department of Radiology, University Hospital, Leiden.

Errata

In the article 'Alteration in Heterocyst Pattern of *Anabaena* produced by 7-Azatryptophan' by G. J. Mitchison and Michael Wilcox (*Nature new Biol.*, **246**, 229; 1973) the diagram captioned 7-azatryptophan shows tryptophan and

not the 7-aza derivative. The nitrogen was inadvertently replaced by a carbon during preparation for the printer.

In the article 'Formation of Lunar Carbide from Lunar Iron Silicates' by C. T. Pillinger *et al.* (*Nature phys. Sci.*, **245**, 3; 1973) Figure 1 legend, lines 4 and 5 should read "³⁶Ar data from Heymann and coworkers⁵⁻⁸ (□) and Bogard and coworkers⁹⁻¹¹ (○): . . .". Paragraph 7, line 9 should read "at least 10² times that necessary for the reaction. Conversely, . . .".

In the article 'Tetrodotoxin and Cobalt Blockade of Neuroblastoma Action Potentials' by Ilan Spectro, Yosef Kimhi and Phillip G. Nelson (*Nature new Biol.*, **246**, 124; 1973) the first line should read 'The mouse C-1300' not 'C-13000'; paragraph 4, line 7 should read 'second, slowly rising peak . . .'; Table 1 legend, line 1 should read MgCl₂ = 1; CaCl₂ = 2 (not MgCl₂ = 2); ref. 5 was published in 1971 not 1972, and the last author in ref. 8 is Nirenberg, M., not Ninberg, M.

Reports and Publications

not included in the Monthly Books Supplement

Great Britain and Ireland

The Natural Environment Research Council. Publications Series B, No. 7: Research in the Geological Sciences. (A consultative document comprising reports from eleven NERC Geological Sciences Working Parties.) Pp. ii+113. (London: The Natural Environment Research Council, 1973.) [111]

The Zoological Record, 1970, Vol. 107, Section 16: Amphibia. Compiled by the Staff of the Zoological Society of London. Pp. vi+79. £6.65. 1970, Vol. 107, Section 17: Reptilia. Compiled by the Staff of the Zoological Society of London. Pp. 90. £6.65. (London: The Zoological Society of London, 1973.) [211]

Scientific Proceedings of the Royal Dublin Society. Series A. Vol. 4, No. 31: Lower Palaeozoic Graptolitic Facies in Ireland and Scotland—Review, Correlation, and Palaeogeography. By J. A. Weir. Pp. 439-460. 75p. Vol. 4, No. 32: Varolitic Lavas from South County Wicklow, Ireland. By J. C. Brindley and S. Millan. Pp. 461-469. 75p. Series B. Vol. 3, No. 13: The Medals of the Royal Dublin Society. By Arthur E. J. West. Pp. 165-190+ plates 1-7. £1. Vol. 3, No. 14: Observations on the Swarming Behaviour of the Polychaete *Platynereis dumerilli* on the West Coast of Ireland. By John P. Mercer and J. Dunne. Pp. 191-194+plate 10. 30p. Vol. 3, No. 15: The Natural Mortality of Cabbage Root Fly Eggs in Peatland. By M. F. Ryan and J. Ryan. Pp. 195-199. 20p. (Dublin: Royal Dublin Society, 1973.) [211]

National Physical Laboratory. Corrosion of Metals Research 1924-1968. By Frank Wormwell. Pp. xix+73. (London: HMSO, 1973.) £1.65 net. [511]